Indirect Effects of Cytomegalovirus

a report by
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Human cytomegalovirus (CMV) is a common human pathogen that infects the majority of the world's population. CMV is a large species-specific virus that co-evolved with its host for many thousands of years. Since CMV adapted to persist in immunocompetent individuals, there has been tremendous evolutionary pressure on the virus to develop mechanisms that enable it to co-exist with its host. The viral genome contains 252 open-reading frames with a potential coding capacity for over 250 proteins. Of these, only approximately 50 are considered essential for viral production. Most CMV proteins are devoted to other tasks. These non-essential viral proteins have evolved sophisticated strategies to affect cellular and immunological functions to help the virus replicate and evade the host immune system. These proteins may also contribute to the pathogenesis of many common diseases.

Direct Effects of Cytomegalovirus Lead to Clinical Disease

Primary CMV infection may produce mild or mononucleosis-like symptoms and thereafter establishes a life-long latency and persistence. For many years, CMV was not considered to give rise to any clinical diseases. As the number of patients with a suppressed immune system has increased, resulting from organ transplantation and AIDS, it has become a major clinical problem. Major efforts have been made to devise clinical diagnostic techniques to detect the virus and to develop antiviral drugs to manage the symptoms.

CMV can infect many cell types and organs. The most common clinical manifestations in immunocompromised patients are CMV syndrome (viraemia, fever, malaise and leucopenia) and organ-specific CMV disease (hepatitis, gastrointestinal disease, pneumonitis and retinitis). Both are considered to be caused by the direct effects of the virus and are likely to be mediated by lytic infection and immune-mediated destruction of cells, leading to acute organ dysfunction. In patients with CMV disease, the virus can be detected in tissues by standard histopathological techniques concurrently with the development of symptoms.

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Indirect Effects of Cytomegalovirus

Once rapid diagnostic tools were developed and CMV infections could be managed with antiviral drugs, life-threatening infections decreased. CMV remains a significant threat, especially in organ and stem cell transplant recipients. Without prophylactic measures, 35–90% of these patients develop clinical CMV infection, generally approximately 40 days after transplantation.

By the 1980s, it was recognised that patients with CMV infection were at an increased risk of complications such as rejection and graft-versus-host disease (both acute and chronic). CMV infections are also associated with cardiovascular disease, opportunistic infections, lymphoma and post-transplant diabetes.[1–6] Since CMV has been difficult to detect in patients or in affected organs, its role in these diseases was thought to be mediated by indirect effects. Viral replication and the onset of disease are often temporally distinct. Prophylaxis against CMV significantly reduces the risk of these indirect effects.

New data may challenge this dogma. Using more sensitive techniques than are available in clinical laboratories, Helaterä et al. found CMV antigens or DNA in 35.4% of kidney graft biopsy samples obtained more than two months after the last positive finding in blood or urine.[7] Patients with persistent intra-graft CMV infection had reduced creatinine clearance and graft survival.

Using an optimised sensitive method for detecting low-grade active CMV infection in organ grafts, we found active low-grade CMV infection in 91 out of 92 grafts in heart transplant patients. When we used a standard staining technique, only 42.5% of the samples were CMV-positive. Sequential biopsies, available for 11 patients, enabled us to examine the relationship between CMV infection and rejection episodes. In all cases, CMV infection was detected before rejection was diagnosed. However, the viral load at the time of rejection was not necessarily high. We suspect that the virus initiates the rejection episode, but by the time rejection is diagnosed, the immune system has cleared the infection and viral levels have decreased. In some cases, inflammation interpreted as a sign of rejection may actually reflect an immune response to an undiagnosed CMV infection.

Cytomegalovirus Is Dependent on Inflammation

As long as CMV is maintained in balance with the host immune system, the infection is most likely to be silent and will not result in protein production or clinical disease.[8] If the virus is reactivated in a patient with a suppressed immune system, it is likely to develop into clinical disease. As CMV infections are common in transplant recipients, immunosuppression was believed to allow the reactivation of latent CMV. Instead, immune activation and inflammation appear to be the

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leading cause of reactivation in transplant patients. The virus establishes latency and persists in monocytes or pre-monocytic cells. Upon allogeneic stimulation of T cells and the production of inflammatory cytokine, latently infected monocytes differentiate into inflammatory macrophages, resulting in reactivation.[9–11] Differentiation of myeloid cells into dendritic cells also results in reactivation.

Reactivation of CMV appears to be cytokine-dependent and linked to the differentiation status of the myeloid cell.[12] Tumour necrosis factor-α (TNF-α) may be particularly important.[13-16] TNF-α is translocated into the nucleus, where it binds to a nuclear factor kappa-B (NF-κB)-binding motif in the enhancer region of the immediate–early (IE) promoter, thereby presumably initiating IE expression in cells of the myeloid lineage.[17,18]

Cytomegalovirus Induces Inflammation

As inflammation is considered to be central to the life-cycle of the virus, it is not surprising that CMV developed mechanisms to induce and maintain inflammation. CMV infection results in an induced production of pro-inflammatory cytokines and chemokines, as well as immunosuppressive cytokines that modulate the host immune response. For example, CMV-infected cells themselves exhibit enhanced production of TNF-α, interleukin (IL)-1β, IL-6, IL-8, IL-10, onconstatin-M, platelet-derived growth factor, basic fibroblast growth factor, monocyte chemo-attractant protein (MCP)-1, regulated on activation normal T-expressed and secreted (RANTES) protein and transforming growth factor-β, all of which may promote inflammation.[8,19] It was recently found that CMV can initiate the production of leukotrienes. These powerful immune molecules are only produced by inflammatory cells such as granulocytes and macrophages, owing to their restricted expression of 5-lipoxygenase (5-LO), the enzyme responsible for catalysing the biosynthesis of leukotrienes from arachidonic acid. In smooth-muscle cells (SMCs), CMV induces 5-LO expression and the production of leukotriene B4.[20] In patients with inflammatory bowel disease, we found 5-LO expression in small-vessel SMCs clearly associated with leucocyte infiltrates.[20] CMV infection also induces cox-2 expression and prostaglandin synthesis.

Cytomegalovirus and Acute Rejection

CMV may induce and maintain inflammation to secure an environment that facilitates viral reactivation and replication. In transplant patients, a rejection episode may initiate reactivation due to an allogeneic response and inflammation. The virus may then further induce and maintain the inflammatory process in the graft. This may explain the association of CMV with acute rejection,[21–23] but it is difficult to define which comes first, as the viral life-cycle is closely linked to inflammation. Prophylaxis against CMV prevents viraemia and reduces the incidence of acute rejection episodes in transplant patients.[22–25] Ganciclovir appears to be more effective than acyclovir,[26,27] and with more aggressive protocols using antiviral drugs and immune globulin, rejection frequency is even lower.[28] In contrast, pre-emptive treatment that prevents clinical CMV infection and organ-invasive disease, but not viraemia, does not appear to affect the risk of rejection,[29,30] perhaps because low-level CMV replication is present and increases the risk of rejection. CMV appears to be intimately involved with inflammation and rejection, and control of viral replication may decrease the risk of these complications after organ transplantation.[31]

Cytomegalovirus, Cardiovascular Disease and Chronic Rejection

Once an infection is established in an organ graft, CMV may use its unique ability to affect cellular and immunological functions to exacerbate chronic inflammation, tissue destruction with fibrosis and vascular re-modelling, leading to impaired graft survival.[8,19,24,31] CMV has been linked to atherosclerosis, arterial restenosis after angioplasty, transplant vascular sclerosis (TVS) and chronic rejection.[31–33] CMV proteins and nucleic acids have been detected in early lesions in diseased vessels, and seropositivity for CMV is closely associated with carotid and coronary artery disease, as well as TVS.[19,32–34] In heart transplant recipients, CMV infection doubles the risk of graft failure during the five-year period immediately after transplantation, as a consequence of the accelerated development of TVS. Prophylactic treatment of such patients with antiviral drugs directed against CMV reduces the risk of developing TVS.[31–35]

Both *in vivo* and *in vitro*, CMV can infect and proliferate within SMCs, macrophages, endothelial cells and fibroblasts – the most important cell types associated with vascular lesions. Such infection disturbs various cellular functions. CMV-induced release of growth factors and cytokines can stimulate the migration of SMCs to the site of vascular injury, and infected cells have an enhanced ability to respond to these stimuli. In addition, examination of human specimens, obtained by atherectomy from patients with and without angioplasty, has revealed that seropositivity for CMV is correlated with inactivation of the tumour suppressor gene p-53.[36] The CMV-encoded IE86 protein has been proposed to mediate this inactivation *in vitro*, thereby promoting the proliferation of infected SMC cells.

The CC and CXC sub-families of chemokines have been detected in atherosclerotic plaques in humans and animal models. CXC chemokines contribute to atherogenesis in mice. The CC chemokine MCP-1 is expressed by endothelial cells, macrophages and SMCs in response to injury and acts as a major mediator of the transendothelial migration of monocytes and T-cells. CMV infection can increase the production of both RANTES and MCP-1.[37,38]

The CMV genome encodes four potential homologues of chemokine receptors: US27, US28, UL33 and UL78. In the presence of RANTES or MCP-1, US28 induces migration of CMV-infected SMCs.[39] Removal of the US28 gene from the viral genome by mutation eliminated SMC migration, which could only be restored by expression of the viral homologue, and not by a cellular G-protein-coupled receptor. US28, the first viral G-protein-coupled receptor shown to mediate the movement of a specific type of cell, provides a plausible molecular explanation for the acceleration of vascular disease processes by CMV. These findings suggest that migration of CMV-infected SMC to sites of vascular injury *in vivo* exacerbates neo-intimal hyperplasia and vessel narrowing. CMV can also alter the metabolism and accumulation of lipids in infected cells by upregulating expression of the scavenger receptor CD36,[40] which may lead to the early development of foam cells and fatty streaks. CMV infection also induces oxidative stress in endothelial cells through increased expression of asymmetric dimethylarginine, the endogenous inhibitor of nitric oxide synthase. Heart transplant recipients have higher plasma levels of this inhibitor than healthy controls and develop more extensive TVS.[41] CMV infection also results in the generation of intracellular reactive oxygen species that activate

NF-κB, a cellular transcription factor involved in expression of both the CMV promoter and genes involved in immune and inflammatory responses. Antioxidants or aspirin inhibit reactive oxygen species, NF-κB and CMV.[42]

CMV may contribute to destabilisation of plaques in established atherosclerotic lesions. We recently found that CMV alters the composition of the intracellular matrix by downregulating matrix metalloprotease-9 and inducing production of the tissue inhibitor metalloprotease-1. In addition, CMV-infected endothelial cells over-express von Willebrand factor (VWF), which enhances platelet aggregation and coagulation and may initiate thrombus formation on infected cells.[43] CMV may also play a role in myocardial infarction.

> It is now crucial to further determine the clinical relevance of low-grade active cytomegalovirus infections in both immunocompetent and immunosuppressed patients.

Cytomegalovirus Infection and Malignancies

CMV is not considered to be oncogenic, but it may have an onco-modulatory role. Sensitive detection techniques have revealed that the CMV genome and proteins are frequently present in colon cancer,[44] malignant glioma,[45,46] prostatic intra-epithelial neoplasia and prostatic carcinoma.[47] The viral infection remains latent in non-cancerous tissue specimens and in healthy controls. We confirmed the presence of an active CMV infection in 99% of malignant glioblastomas and found that the infection level in the tumour correlates strongly with time to tumour progression and survival. CMV-infected tumour cells may escape immune surveillance.[8,48,49] The virus can then affect cellular differentiation, cell-cycle regulation, epigenetic functions, angiogenesis, migration and, through anti-apoptotic proteins, confer resistance against conventional chemotherapy.[8,50,51] These strategies may, in many different ways, lead to progression of cancer. Whether CMV infection is truly causative or is simply an epiphenomenon of malignant tumours requires further elucidation.

Cytomegalovirus-induced Immunosuppression and Increased Risk of Concomitant Infections

CMV severely impairs several key functions of the immune system.[48,52] CMV inhibits the monocyte differentiation in the antigen-presenting cells macrophages and dendritic cells.[48,52] It can also impair the maturation and migration of dendritic cells and inhibit T-cell proliferation. Four CMV proteins downregulate the expression of HLA class-I molecules, impairing the ability of cytotoxic T cells to kill infected target cells.[48,52] Through several mechanisms, it down-regulates expression of human leukocyte antigen (HLA)-class-II and weakens the T-helper-cell response.[48,52] CMV infection of plasma-cytoid dendritic cells also dampens the T-cell response.[53]

Several CMV-specific mechanisms control the activation of natural killer (NK) cells,[48–52] and the virus confers protection against the action of perforin and granzyme-B released by activated T cells and NK cells.[54] These mechanisms impair the host's ability to combat other infections. Meta-analyses demonstrate that prophylaxis against CMV decreases the risk of bacterial and protozoal infections by 35 and 69%, respectively.[1,4] CD4 and CD8 T cells are critical for the control of viral replication. The CMV-pp65 protein is a major target of CD8 T cells. T cells react to many other CMV antigens.[55] IE-specific CD8 T cells appear to correlate with protection against CMV infection in heart and lung transplant patients.[56] In particular, the proportion of CD8 T cells that secrete interferon gamma appears to be important in controlling asymptomatic viraemia.[57] T-cell activation, in response to sub-clinical CMV infection in heart transplant patients, prevents CMV viraemia, acute rejection and coronary artery lumen loss.[58] These observations imply that efficient immunological control of CMV offers protection against both the direct and indirect effects of CMV.[31]

In summary, CMV proteins have developed sophisticated mechanisms that enable the virus to co-exist with its host. These proteins may contribute to the pathogenesis of chronic inflammatory diseases and cancer. It is now crucial to further determine the clinical relevance of low-grade active CMV infections in both immunocompetent and immunosuppressed patients. Optimal management of CMV infection is especially important in transplant patients. ■

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