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Review

Inhibition of cytomegalovirus immediate early gene expression: a therapeutic option?

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Abstract

The replication cycle of the human cytomegalovirus (HCMV) is characterized by the expression of immediate early (IE), early (E), and late (L) gene regions. Current antiviral strategies are directed against the viral DNA polymerase expressed during the early phase of infection. The regulation of the IE-1 and IE-2 gene expression is the key to latency and active replication due to their transactivating and repressing functions. There is growing evidence that the pathogenic features of HCMV are largely due to the abilities of IE-1 and IE-2 to transactivate cellular genes. Consequently, current drugs used to inhibit HCMV infection would have no impact on IE-1 and IE-2-induced effects that are produced before the early phase. Moreover, when HCMV DNA replication is inhibited, IE gene products accumulate in infected cells causing disturbances of host cell functions. This review summarizes the biological functions of HCMV-IE gene expression, their relevance in pathogenesis, as well as efforts to develop novel treatment strategies directed against HCMV-IE expression.

Keywords: Cytomegalovirus; Immediate early gene expression; Immunopathomechanisms; Antiviral therapy

1. HCMV infection: clinical implications and therapy

1.1. Clinical implications of HCMV infection

HCMV infection is the most common infectious complication in immunosuppressed patients after solid organ and bone marrow transplantation. Besides transplant recipients, other immuno-

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compromised patients such as transfusion patients, leukemia patients, and AIDS patients belong to the HCMV high risk group.

Immunosuppression prevents antiviral immune control mechanisms and leads to unimpaired HCMV replication (Reddehase et al., 1987) which may result in the cytomegalic inclusion disease (CID) with destruction of the infected tissue. On the other hand, in spite of systemic immunosuppression, HCMV-associated inflammatory immune reactions may contribute to diseases such as transplant rejection, graft versus host disease, retinitis, pneumonitis, colitis even without detectable virus replication in the respective tissues (reviewed in Scholz et al., 1998). In these cases, treatment of the disease with antiviral drugs targeted against viral DNA replication will be without success.

1.2. Current anti-cytomegalovirus treatment strategies and available drugs

Antiviral treatment strategies can be divided into four major categories: (a) *prevention* of infection; (b) *prophylactic* therapy without evidence for virus replication; (c) *preemptive* therapeutic suppression of virus replication in the absence of disease; and (d) *treatment* of overt disease (Scholz et al., 1997a; Vogel et al., 1998). In this review the focus will be on the treatment of overt disease, which is relevant in immunocompromised patients such as transplant recipients and AIDS patients. In general, the drugs available for anti-HCMV therapy are limited. To date, the nucleoside analog ganciclovir (GCV), the nucleotide analog cidofovir (CDV), and the pyrophosphate analogue foscarnet (FOS) have been approved. In addition, an antisense oligonucleotide against HCMV IE2 mRNA (ISIS 2922, fomivirsen, Vitravene) has been developed (Azad et al., 1993; Anderson et al., 1996) for intravitreal application in HCMV retinitis patients (De Smet et al., 1999). The application of this drug is restricted to patients who do not respond to other HCMV therapies. Since GCV has turned out to be superior over aciclovir, GCV normally is used as the first line medication. The major severe side effect of GCV is the myelotoxicity manifesting as neutropenia. Moreover, GCV maintenance therapy may lead to HCMV resistance due to mutations in the UL97 (phosphotransferase) and UL54 (viral DNA polymerase) genes (Chou et al., 1997). On the other hand, FOS is mostly used as an alternative to GCV (e.g. in case of GCV resistance or severe side effects). Side effects such as nephrotoxicity and disturbance of the electrolyte balance limit the application of FOS (as reviewed by Vogel et al., 1998). Moreover, long-term application may lead to FOS resistance due to UL54 mutations (Knox et al., 1991). Finally, CDV (an acyclic nucleotide analogue; Vistide) has been approved for the treatment of HCMV retinitis in the United States, Europe and elsewhere (De Clercq, 1998). It is known that CDV treatment may be associated with nephrotoxicity, electrolyte imbalance, and resistance due to UL54 mutation, the latter following preceding treatment with GCV (Hitchcock et al., 1996; Jabs et al., 1998; Cherrington et al., 1998; Cihlar and Hitchcock, 1998).

The modes of action of the above compounds have been described in several communications (Balzarini et al., 1998; Field, 1999). Both, GCV and CDV are known to be competitive inhibitors of DNA polymerase whereas FOS non-competitively inhibits HCMV DNA polymerase by binding close to the pyrophosphate binding site. All three drugs result in significant reduction of the rate of HCMV DNA synthesis. In addition to the above described compounds, lobucavir is another nucleoside analogue that inhibits HCMV DNA synthesis by a mechanism that is comparable to that of GCV. However, lobucavir has not been further developed because of its possible carcinogenic effects.

Other antiviral strategies have been reviewed by Field (1999). For example, inhibition of gB processing with a bioengineered serpin, alpha1-PDX (Jean et al., 1998, 2000) and inhibition of the HCMV serine protease (UL80 gene product) with protease inhibitors (Shieh et al., 1997) have been evaluated experimentally. Other promising new anti-HCMV compounds are the benzimidazoles such as BDCRB or 1263W94 both acting via a unique mechanism distinct from the inhibition of DNA polymerase. 1263W94 is now being evaluated in the clinical setting in HIV-infected subjects

with asymptomatic HCMV shedding (Chulay et al., 1999).

Another promising antiviral strategy is the inhibition of HCMV IE gene products by a phosphorothioate oligonucleotide, GEM 132 (UL36 ANTI). UL36 ANTI is complementary to the splice donor site of the IE gene UL36 and therefore inhibits HCMV DNA origin of replication-dependent synthesis (Smith and Pari, 1995; Pari et al., 1995).

Yet another phosphorothioate oligonucleotide, ISIS 2922, is complementary to the mRNA encoding the major polypeptide products of the IE region 2 (*ie*2). (Anderson et al., 1996; Azad et al., 1993). Treatment of normal human fibroblast cells with ISIS 2922 results in reduction of not only IE-2 levels but also in sequence-independent reduction of IE-1 levels (Anderson et al., 1996; Cinatl et al., 1999a, 2000a). However, since cells were treated with ISIS 2922 prior to infection with HCMV, non-specific effects of ISIS 2922 due to inhibition of HCMV adsorption and/or penetration cannot be excluded. Indeed, such non-specific antiviral effects were previously observed when ISIS 2922 was employed at doses greater than 1 μM (Anderson et al., 1996).

2. Biological functions of HCMV IE proteins

The sequential expression of the HCMV genome has been divided into three time phases: α ('immediate early'; IE), β1 and β2 ('delayed early'; DE), γ1 and γ2 ('late'; L) based on the appearance of the respective mRNA or protein (Stinski et al., 1991). The α gene expression is essential for the switch from α to β and partly γ gene expression and thus for the progression of the replication cycle. It has been suggested, therefore, that both latent infection and reactivation are determined by the activity of IE gene products. The most abundantly expressed *ie* gene products IE-1 and IE-2 were first shown by Everett and Dunlop (1984) to exhibit transactivation properties. Thus, important aspects of the regulation and the biological function of the *ie*1 and *ie*2 gene regions are summarized below.

The transcription of *ie*-1 and *ie*-2 gene regions is controlled by the major *ie* promoter-regulatory region. In permissive human foreskin fibroblasts (HFF), IE mRNA and/or proteins can be detected 2–4 h post-inoculation (p.i.) in vitro. Thomsen et al. (1984) showed that transcription from the HCMV major IE promoter may be inhibited by α-amanitin suggesting that IE is transcribed by RNA polymerase II of the host cell.

The *ie* gene products not only have autoregulatory features but also are strong *trans*-activators, known to stimulate the transcription of various viral and host cell genes, e.g. by protein–protein and/or protein–DNA interactions (Winkler et al., 1994; Arlt et al., 1994; Lang et al., 1995; Meier and Stinski, 1996; Romanowski et al., 1997; Romanowski and Shenk 1997; Wang et al., 1997; Wu et al., 1998; Bruening et al., 1998; Murphy et al., 2000; Winkler et al., 2000; Lundquist et al., 1999). The major immediate early (*ie*-1/*ie*-2) promoter–enhancer is located upstream of the *ie*-1/*ie*-2 locus and contains multifold binding sites for viral (e.g. IE-2, pp71 tegument protein) and cellular transcription factors such as NF-κB, ATF/CREB, AP1, SP1, C/EBP, SREB, and NF1 (Ghazal et al., 1987, 1988a,b, 1990; Nelson et al., 1990; Sambucetti et al., 1989; Zhang et al., 1991). Some of these cellular transcription factors bind to repetitive elements (17-, 18-, 19-, and 21-bp motifs). For example, in immunocompromised patients such as transplant patients, aberrant secretion of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) may result in cytokine-mediated activation of NF-κB and subsequent binding of NF-κB to the 18-bp sequence motif of the major *ie* promoter–enhancer (as discussed in detail below). On the other hand, IE-1 is autostimulatory via activation of NF-κB, which in turn stimulates the major *ie* promoter–enhancer and thus expression of *ie*-1 (Sambucetti et al., 1989; Cherrington and Mocarski, 1989). Particular *ie*-2 gene products act as negative autoregulators by binding to the *cis*-repression signal (crs) sequence located immediately upstream of the *ie*-1/*ie*-2 transcription start site (Cherrington et al., 1991; Hermiston et al., 1990; Liu et al., 1991; McKeating et al., 1990; Pizzorno et al., 1988; Stenberg et al., 1990). Therefore, *ie*-2 gene products, especially IE-2_{579aa} and IE-2_{338aa}

(late *ie*-2 gene product), mediate the shut-off of *ie*-1 and *ie*-2 gene expression probably at the transcriptional level by altering the RNA polymerase II (Wu et al., 1993).

However, IE-2 proteins alone or in synergism with IE1 may be promiscuous transactivators of both viral and cellular gene expression. Transactivation of delayed early and late genes by *ie*-2 gene products seems to be an essential step in the lytic replicative cycle during productive infection (Everett and Dunlop, 1984; Pizzorno et al., 1988; Tevethia et al., 1987; Hermiston et al., 1990; Burns et al., 1999). It has recently been demonstrated that a viral early protein inhibits the IE-2-p86-mediated transactivation of homologous and heterologous promoters and, in addition, augments its negative autoregulation (Gebert et al., 1997). This inhibition of IE function by early proteins may be an important mechanism that accounts for persistent infection and latency.

In contrast to the major *ie* gene products, several ancillary IE proteins (reviewed by Colberg-Poley, 1996), encoded by the HCMV UL36-38, UL115-119, TRS1/IRS1 and US3 loci, are type I integral membrane *N*-glycoproteins. However, these proteins are known to have transactivating properties and to regulate nuclear gene expression. For example, proteins encoded by the UL36-38 have been reported to be important for virus growth in fibroblasts (Smith and Pari, 1995; Pari et al., 1995). These *ie* gene products may have direct effects on cellular metabolism or may influence cellular gene expression. For example, individual UL36, UL37x1, or UL37 IE proteins can regulate expression of the hsp70 protein (Colberg-Poley et al., 1992). A product of the UL37 gene can inhibit apoptosis induced by different stimuli (Goldmacher et al., 1999). US3 products have been shown to retain the MHC class I heavy chains in the endoplasmic reticulum and thus prevent antigen presentation of viral products on the host cell membrane (Jones et al., 1996; Ahn et al., 1996). It should be mentioned that some virus-encoded transactivating proteins such as TRS1 or UL82 (pp71) readily enter the cell with the virus and thus even inhibition of major IE proteins may not completely prevent HCMV disorders (Romanowski et al., 1997; Bresnahan and Shenk, 2000a).

3. The role of HCMV IE in pathogenesis

3.1. Immunomodulation

Grundy et al. (1987) reported that HCMV pneumonitis in allogeneic transplant recipients did not correlate with direct viral cytopathogenicity and thus proposed an immunopathological origin of the disease. Moreover, many reports exist, discussing the role of HCMV in modulating the pro-inflammatory activity of the immune system (Grundy, 1998). Along these lines, HCMV retinitis in AIDS patients was proposed to be partly due to immunopathological effects induced by HCMV IE because disease progression was found in the absence of replicating virus (Jacobson et al., 1997; Gümbel et al., 1998). Similarly, in a mouse model, Tanaka et al. (1994) obtained immunologically induced MCMV pneumonitis without inducing active virus infection. In a small number of HCMV retinitis patients, disease progression could be halted after treatment with the antiviral and immunomodulatory metal chelator desferal (DFO), whereas treatment with GCV and/or FOS failed to do so (Gümbel et al., 1998). Interestingly, in cultured human umbilical vein endothelial cells, DFO suppressed cytokine-stimulated intercellular adhesion molecule-1 (ICAM-1; Cinatl et al., 1995a). It was therefore suggested that the beneficial effect of the chelator was due to the inhibition of HCMV IE-induced immunopathological events (Scholz et al., 1997b). Whereas DFO and another metal chelator, diethylentriaminepentaacetic acid, failed to inhibit rat CMV (Kloover et al., 1999), liver transplant rejection was reduced by DFO, as determined histologically (Martelius et al., 1999). Since DFO has been shown to limit inflammatory immune reactions in vitro (Scholz et al., 1996a) and autoimmune reactions in vivo (Blake et al., 1983) this agent may be of clinical importance in the treatment of graft recipients. However, mycophenolate mofetil, a novel immunosuppressive drug, was recently shown to increase HCMV invasive organ disease in renal transplant recipients (Sarmiento et al., 2000).

As already mentioned, HCMV-mediated inflammation may result in disturbance of the im-

munological balance within the respective tissue (Scholz et al., 1995, 1997c). HCMV-infected cells may be protected by virus-induced inhibition of apoptosis (Zhu et al., 1995; Cinatl et al., 1998; Goldmacher et al., 1999). Thus, the quiescent non-replicating virus within the latently infected cell may employ the pro-inflammatory activity of the immune system to become activated and/or to disseminate. Consequently, the status of whether HCMV infection leads to allograft rejection or whether HCMV becomes activated by the overshooting immune responses during graft rejection crises is not fully understood. However, in our view both pathways may occur concomitantly and HCMV-associated disease may be due to very complex and overlapping viral and immune pathways.

3.2. Adhesion and transendothelial migration

It seems that HCMV triggers the intercellular interactions involved in transendothelial migration distinctively at the molecular level (Scholz et al., 2000). Adhesion of leukocytes to endothelial cells and the subsequent transendothelial migration are prerequisites of tissue infiltration. It has been reported by our group (Scholz et al., 1992) and by others (Sedmak et al., 1994) that HCMV-infected endothelial cells (EC) exhibit augmented expression of ICAM-1, which is the ligand of leukocyte function associated-1 (LFA-1) on leukocytes. The interaction of ICAM-1 and LFA-1 is critical for the leukocyte/EC interactions. In our cell culture studies inhibition of HCMV with GCV prevented virus DNA replication but also enhanced HCMV-induced ICAM-1 expression suggesting that this virus-induced effect is initiated during the IE phase of viral replication. Similar findings have been reported by Craigen and Grundy (1996). Indeed, HCMV-induced ICAM-1 mRNA and protein expression could be prevented by pretreatment of the cells with the antisense oligonucleotide ISIS 2922 which effectively inhibits the IE mRNA of HCMV. Recently, Burns et al. (1999) proposed that HCMV IE proteins directly stimulate the heterologous ICAM-1 gene promoter. In transient cotransfection experiments with ICAM-1 promoter plasmid p-1165CAT and plasmids containing the HCMV major ie promoter upstream of the IE-1, IE-2, or IE-1 and IE-2 coding regions, these authors showed that IE-2 but not IE-1, induced ICAM-1 expression. The combination of IE-1 and IE-2 revealed synergistic effects.

In addition to the HCMV-mediated enhancement of ICAM-1 expression on EC, augmented adhesion and transendothelial migration of leukocytes was found. Grundy and Downes (1993), Grundy et al. (1993) obtained evidence that HCMV-induced LFA-3 and ICAM-1 resulted in either enhanced binding of resting CD2+ lymphocytes or activated lymphocytes. Despite these findings, the role of HCMV-induced ICAM-1 expression needs to be further evaluated. In coculture experiments with allogeneic lymphocytes, HCMV-mediated upregulation of ICAM-1 was observed on EC, but was not the reason for enhanced transendothelial migration, since ICAM-1 expression on the surface membrane of EC occurred later than lymphocyte migration (Blaheta et al., 1994). In this regard, it may be speculated that ICAM-1 is not primarily important for the virus during the early phase of infection but rather in the late phase of infection when adherent leukocytes can be used as a virus carrier for dissemination throughout the body via the blood stream. In cultured human retinal pigment epithelial cells (RPE) HCMV infection similar to the EC results enhanced ICAM-1 expression. However, neutrophils exhibit reduced adhesion to HCMV-infected RPE, and this suggests that other HCMV-induced mechanisms control adhesion with higher priority over ICAM-1 (Cinatl et al., 2000b).

3.3. Modulation of pro-inflammatory cytokines/chemokines

HCMV infection has been associated with pro-inflammatory immune responses such as cytokine/chemokine production and secretion by immunorelevant cells. Many of these HCMV-induced cytokines/chemokines are produced despite the inhibition of viral DNA replication. More specifically, it has been demonstrated that viral *ie* gene expression is associated with upregulation of

cytokine gene expression. For example, Geist et al. (1994) found by transient transfection assays in the myelomonocytic cell line THP-1 that *ie* genes activated the TNF-α promoter and increased mRNA and protein expression. Moreover, the site within the TNF-α promoter where the HCMV *ie*-1 gene product mediates its effect was determined. Additionally, it was shown that HCMV infection of human alveolar macrophages alters the binding activity of transcription factors that in turn regulate TNF-α gene expression (Geist et al., 1997). Enhanced TNF-α serum levels were shown to be associated with inflammatory diseases such as chronic liver disease and B cell chronic lymphocytic leukemia, allograft rejection and also septicemia (Mutimer et al., 1997; Kutza et al., 1998). TNF-α is a strong immunomodulator and acts via binding to the TNF-α receptor on the target cells. This binding causes the activation of the pleiotropic transcription factor NF-κB which is then translocated into the nucleus. The expression of numerous immunomodulatory molecules (e.g. adhesion molecules ICAM-1, E-selectin, vascular cell adhesion molecule-1; VCAM-1) but also promoters of viruses such as HIV and HCMV are regulated by NF-κB (Roulston et al., 1995; Prösch et al., 1995, 1998). Moreover, HCMV directly activates NF-κB and other transcription factors at early times of infection (for review see Fortunato et al., 2000).

The effects of HCMV on other pro-inflammatory cytokines have been investigated by several groups. Geist and Dai (1996) demonstrated that HCMV infection increased IL-6 protein and mRNA in peripheral blood mononuclear cells. In addition, HCMV *ie*-1 gene products increased expression of the IL-6 promoter. Elevated IL-6 levels were proposed to be induced by HCMV independently of active infection (Carlquist et al., 1999) and associated with lung transplant rejection or infection and may be used as a predictive marker (Yoshida et al., 1993). In bone marrow transplant recipients elevated IL-6 serum levels were recently found to be associated with HCMV disease (Humar et al., 1999). The deregulated expression of IL-6 and its receptor is involved in a variety of diseases (reviewed by Hirano, 1998), including disorders of the central nervous system (Gruol and Nelson, 1997). HCMV-induced enhancement of IL-6 expression may control multiple signal transduction pathways which in turn regulate the expression of several genes including c-myc, c-myb, junB, IRF1, egr-1, and bcl-2. These genes are involved in the regulation of cell growth, differentiation and apoptosis (Hirano, 1998).

In vitro transfection studies revealed that HCMV IE-1 + 2 upregulates IL-2, the IL-2 receptor (R), IL-1β, and the IL-1 receptor antagonist (IL-1ra) promoter (Geist et al., 1991; Crump et al., 1992; Hunninghake et al., 1992; Kline et al., 1994; Geist and Dai, 2000). Interestingly, modulation of these cytokines was differentially achieved by HCMV IE-1 and IE-2. HCMV IE-2, but not IE-1, upregulated IL-2 and IL-2Rα promoter activity and the respective mRNA levels. In solid organ transplant recipients, upregulation of IL-2 as well as IL-2R may be extremely critical because induction of cell-mediated immune responses could lead to allograft rejection crises. Cyclosporin A, one of the major immunosuppressive agents used for the prevention of allograft rejection, successfully inhibited IL-2 production. However, when Jurkat T cells were transfected with plasmids expressing the HCMV *ie*-2 gene products, the inhibitory effect of cyclosporin A on IL-2 promoter activation and gene transcription was abolished (Geist et al., 1992). Thus, HCMV IE-2 expression might neutralize the iatrogenic immunosuppression which is required in transplant recipients and may enhance inflammatory mechanisms, e.g. in autoimmune diseases such as rheumatoid arthritis (Stahl et al., 2000). Furthermore, primarily HCMV IE-1, but not HCMV IE-2, up-regulated the expression of the IL-1β gene (Crump et al., 1992). However, HCMV IE-2 protein was shown to stimulate interleukin 1β gene transcription via tethering to Spi-1/PU.1 (Wara-aswapati et al., 1999). IL-1 is a prototypic pro-inflammatory cytokine that is also involved in autoimmune diseases such as insulin-dependent diabetes mellitus (reviewed by Reimers, 1998). However, *ie*-1 requires the presence of the transcription factor NF-beta A in order to transactivate the proximal IL-1β promoter (Hunninghake et al., 1992). Furthermore, HCMV IE-1 down-

regulates IL-1ra that binds to IL-1 receptors but does not induce any intracellular responses. Thus HCMV IE-1 augments the pro-inflammatory activity of IL-1. Interestingly, HCMV IE-2 was found to upregulate IL-1ra gene expression and thus may act as a counterpart of HCMV IE-1-mediated inflammation (Kline et al., 1994).

In addition to the indirect pro-inflammatory impact of HCMV via triggering cytokine expression, recent evidence is accumulating that HCMV IE products manipulate the immune system by attracting granulocytes. It has been proposed that HCMV may escape the immune system by US28 expression that results in sequestration of the chemokines RANTES and MCP-1 (Bodaghi et al., 1998; Billstrom et al., 1999). However, the recent publication of Hirsch and Shenk (1999) indicates that HCMV-mediated down-regulation of MCP-1 is US28-independent. More importantly, evidence has been presented that, opposite to in vitro observations, MCP-1 is elevated in patients infected with HCMV (Bernasconi et al., 1996; Nordoy et al., 2000). Several groups were able to show that HCMV infection results in enhanced or de novo-induced secretion of α-(C–X–C) and β-(C–C) chemokines (reviewed in Scholz et al., 1998). In the human monocytic cell line THP-1 and in the astrocytoma cell line U373MG, HCMV was shown to induce IL-8 gene transcription which was dependent on the activation of AP-1 and NF-κB transcription factors (Murayama et al., 1997, 2000). These effects were shown to be mediated by HCMV-IE in U373MG and, therefore, are consistent with our own findings in human fibroblasts (Cinatl et al., 2000a). Craigen et al. (1997) observed enhanced neutrophil transendothelial migration after treatment with supernatants of HCMV-infected fibroblasts due to upregulation of IL-8. Grundy et al. (1998) and Revello et al. (1998) demonstrated that HCMV-infected endothelial cells in addition secrete enhanced levels of GRO-α that functionally recruits neutrophils. The HCMV-induced neutrophil/endothelial cell interaction was suggested to be important for virus dissemination. Extensive in vitro studies revealed that standard antiviral drugs directed against HCMV DNA replication failed to inhibit HCMV-induced chemokine secretion and/or expression of adhesion molecules in several cell types leading to the suggestion that *ie* gene products are responsible for transactivation of cellular pro-inflammatory gene expression (Craigen and Grundy, 1996; Michelson et al., 1997; Scholz et al., 1998). Recently, we were able to partly confirm this by demonstrating the inhibition of HCMV-induced IL-8 secretion and function with the *ie* mRNA antisense oligonucleotide ISIS 2922 (Cinatl et al., 2000a). This finding might be important in the prevention of HIV/HCMV coinfection because IL-8 is required for HIV gp120-mediated HCMV reactivation (Capobianchi et al., 1997).

It has been proposed that stress is also involved in HCMV-induced inflammation as well as in inflammation-associated HCMV reactivation (Vossen et al., 1997). Recently, reactivation and shedding of HCMV was reported in astronauts during spaceflight with significant increases of the stress hormones epinephrine and norepinephrine after landing (Mehta et al., 2000). Prösch et al. (2000) showed earlier that these catecholamines stimulated the HCMV *ie* enhancer/promoter. Different stress factors as well as HCMV glycoproteins may activate mitogen-activated protein kinases (MAPKs) such as p38 which are important for up-regulation of the HCMV *ie* enhancer/promoter (Bruening et al., 1998; Boyle et al., 1999). Since chemokines attract neutrophils to the site of infection during the inflammatory processes, it is conceivable that reactive oxygen intermediates (ROIs) may arise intracellularly following oxidative burst. These ROIs are known to activate NF-κB that in turn stimulates the major *ie* promoter/enhancer of HCMV (Prösch et al., 1995) as well as many other promoters. In this regard, it has been shown that oxidative stress upregulates HCMV IE protein expression in HCMV AD169-infected cultured EC (Scholz et al., 1996b) and other cell types (Scholz et al., 1998). Different antioxidants were shown to possess antiviral activity (Cinatl et al., 1995b,c; Vossen et al., 1997). Therefore, the combination of ISIS 2922 with antioxidants such as *N*-acetylcysteine (NAC) has been evaluated. In these experiments H_2O_2-induced IL-8 secretion by HCMV-infected fibroblasts was inhibited by

NAC/ISIS 2922 but not by either drug alone (Cinatl et al., 1999a). The interdependent relation of ROIs and HCMV seems to be similar in smooth muscle cells. In this regard, Speir et al. (1996) reported that in these cells IE72 is important for the induction of ROIs and in turn these ROIs are used for the production of IE72. In contrast to our own studies with endothelial cells, fibroblasts and retinal epithelial cells, Speir et al. (1996) were able to inhibit H_2O_2-induced IE72 production with NAC. On the other hand, IE72 and IE84, at least partially, induced ROIs via transactivation of the cyclooxygenase promoter, a pathway that can be blocked by aspirin and indomethacin (Speir et al., 1998). Furthermore, it has been demonstrated that HCMV infection at IE times of infection stimulates the arachidonic acid cascade via a G protein-dependent signaling pathway including activation of MAPK and cytosolic phospholipase A_2 ($cPLA_2$) phosphorylation. The stimulated arachidonic acid cascade generates intracellular ROIs and activates NF-κB (Shibutani et al., 1997).

3.4. Angiogenesis

Angiogenesis is strongly associated with the development of tumor metastases and with inflammation. HCMV infection has been shown to be involved in the modulation of angiogenic pathways (Alcami et al., 1991). Conditioned cell culture medium harvested from HCMV-infected fibroblasts was shown to induce endothelial cell growth in the absence of recombinant growth factors (Cinatl et al., 1996). Moreover, St. Jeor et al. (1993) were able to demonstrate that the HCMV-induced bFGF production was dependent on HCMV IE expression. It has been further observed that HCMV *ie* gene expression impairs the production of thrombospondin-1 (TSP-1) which may function as a negative regulator of angiogenesis (Cinatl et al., 1999b, 2000c). The HCMV gene UL146-encoded protein vCXC-1 has been shown to be a functional chemokine which contains a ELRCXC motif (Penfold et al., 1999). Chemokines that contain the ELR motif are potent promoters of angiogenesis (reviewed by Belperio et al., 2000). However, the implication of HCMV IE expression in the secretion of ELR + chemokines remains to be investigated.

3.5. Tumor suppressor gene products

Several viruses are known to trigger the cell cycle of their host cells. Although HCMV is not recognized as a tumor virus it does contain morphological transforming regions (mtrI, mtrII, and mtrIII) which have been shown to transform rodent cells in culture (Thompson et al., 1994) and down-regulate p53-activated transcription (Muralidhar et al., 1996). HCMV *ie* gene products as well as products of morphologic transforming region II (mtrII) were found to modify the function of tumor suppressor proteins (as reviewed by Cinatl et al., 1996 and by Doniger et al., 1999; Muralidhar et al., 1996; Bonin and McDougall, 1997; Shen et al., 1997; Lukac and Alwine, 1999; Wang et al., 2000). HCMV IE2-86-kDa binds to p53 and Rb (Speir et al., 1994; Hagemeier et al., 1994) and cooperates with IE1 and adenoviral E1A to transform primary baby rat kidney cells (Shen et al., 1997). Recently, the interaction of IE2 with the Wilm's tumor suppressor, WT1, has been demonstrated (Kim et al., 2000). However, it has been recently reported that dysfunction of p53 was not associated with effects of IE proteins (Bonin and McDougall, 1997; Wang et al., 2000). With regard to HCMV-associated atherogenesis Melnick et al. (1994) have shown that mtrII of HCMV is found in 90% of patients. HCMV IE84 seems to play a crucial role in atherosclerosis and restenosis by interacting with p53 in smooth muscle cells (SMC). This interaction may result in the accumulation of apoptotic SMC or aberrant proliferation both contributing to stenosis of blood vessels (Speir et al., 1994; Zhou et al., 1999). HCMV-associated atherogenesis has recently been reviewed by Bruggeman et al. (1999).

4. Inhibition or modulation of HCMV IE as a therapeutic option?

This review underlines the importance of

HCMV *ie* gene expression in pathogenesis and the need for the development of better IE targeting compounds. In the light of the recent observations reported by Bresnahan and Shenk (2000b) that functional HCMV RNA (including a 5-kb *ie* RNA) may be delivered by infectious virions to the host cell upon infection, the therapeutic limitations of viral DNA inhibitors should be considered. Experimental evidence has shown that ISIS 2922 can impair IE-mediated effects such as pro-inflammatory immune mechanisms. However, ISIS 2922 may largely act in a non-specific way (De Clercq, 1999). Another phosphorothioate oligonucleotide against UL36, GEM132, has not been approved for clinical use yet. However, this compound does not target the major IE transcripts. It may also be possible to inhibit cellular transcription factors or upstream regulator molecules which are required for transcription of HCMV IE proteins. However, these strategies may have a negative impact on cellular metabolism. It is conceivable that highly sophisticated strategies with small molecules may specifically prevent/impair the transactivating properties of HCMV IE proteins. Possible targets might include protein/protein or protein/DNA interactions (Shipps et al., 1997; Seidel et al., 2000) and RNA (Wilson and Li, 2000). In this regard, HCMV IE-associated molecular interactions could be proposed as targets for small antiviral molecules. The identification of important binding domains (Fortunato et al., 1997), phosphorylation sites (Harel and Alwine, 1998) and structural conformations (Wara-aswapati et al., 1999) is essential to develop these inhibitors. One possible intervention early after virus infection of the cell might be during deposition of the viral genome at the so-called promyelocytic leukemia protein (PML)-oncogenic domains (PODs) (Ishov et al., 1997; Ahn and Hayward, 1997). Only these POD-associated genomes express IE transcripts. The newly synthesized IE1 protein may disperse PML from the PODs, a mechanism that is believed to be associated with increased transcriptional activity of cellular genes and thus with increased efficiency of viral gene expression (Ahn and Hayward, 1997; Ahn et al., 1998; Wilkinson et al., 1998; Müller and Dejean, 1999). In fact, the prevention of POD disruption by IE-1 in cells constitutively overexpressing PML at very early times after infection considerably enhanced IE2-p86 co-localization with PODs in association with decreased transcriptional activation of viral early or late promoters, probably due to decreased transactivation activity of IE2-p86 protein (Ahn and Hayward, 2000). Moreover, cellular ubiquitin-homologous peptides, including SUMO-1 and hSMT3b, which are covalently coupled to POD proteins, may also covalently bind HCMV IE2-p86. This modification of IE2-p86 plays a role in IE2-mediated transactivation (Hofmann et al., 2000). It has further been shown that the HCMV IE2-p86 has the ability to directly interact with cellular promoter regions (Bresnahan et al., 1998), resulting in transcriptional activation. The ability of IE2-p86 to bind several cellular factors (Caswell et al., 1996; Furnari et al., 1993; Hagemeier et al., 1992; Jupp et al., 1993b; Lang et al., 1995; Lukac et al., 1994, 1997; Schwartz et al., 1996; Sommer et al., 1994; Speir et al., 1994; Yoo et al., 1996) suggests an important role in bridging between upstream transcription factors (CREB/ATF) and the transcription preinitiation complex. In addition, basal promoter elements containing only a TATA motif can be directly activated by IE2-p86 that interacts with transcription factors such as TFIIB and TFIID (Caswell et al., 1993; Jupp et al., 1993a; Sommer et al., 1994). Recently, Bryant et al. (2000) found that IE2-p86 interacts directly with the histone acetyltransferase P/CAF involved in chromatin configuration towards a transcriptionally active form. An additional interesting approach is the study of the mechanisms by which the viral early proteins inhibit IE functions (Gebert et al., 1997). This may allow us to obtain functional/structural information, which may guide or facilitate the rational design of IE inhibitors.

In conclusion, the development of novel strategies to inhibit or modulate HCMV IE expression and/or function, and the interaction of HCMV IE with PODs, cellular transcription fac-

tors, and promoter regions, is an important goal to treat HCMV-associated diseases that do not respond to the currently used inhibitors of viral DNA replication such as GCV, FOS or CDV.

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