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Antiviral Research 43 (1999) 191–200

Antiviral Research

www.elsevier.com/locate/antiviral

Cytomegalovirus and atherogenesis

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Received 13 April 1999; received in revised form 14 April 1999; accepted 21 May 1999

Keywords: Cytomegalovirus; Atherogenesis; Atheroxclerosis; chronic infection; Latency

1. Introduction

Cytomegalovirus (CMV) establishes a chronic infection in normal (immuno competent) hosts characterized by latency and intermittent shedding of infectious virus. Reactivation occurs mostly in situations of severe immunocompromise such as in transplant recipients treated with immunosuppressive drugs or in AIDS patients.

Since several years there is a accumulating evidence that certain microorganisms play a role in the pathogenesis of vascular pathology (Libby et al., 1997; Kol and Libby, 1998). An infectious cause of atherosclerosis fits into the generally accepted response-to-injury model of atherogenesis (Ross 1993).

A viral role in atherosclerosis was proposed for the first time in the seventies by Fabricant et al. (1978). There was also circumstantial evidence that besides Marek's disease virus other herpesviruses such as herpes simplex virus (HSV), and especially CMV, can contribute to the pathogenesis of atherosclerosis (Bruggeman and van Dam-Mieras 1991). The strongest evidence of the pathogenic role of CMV in vascular disease is found in transplant associated arteriosclerosis and endothelialitis occurring in cardiac transplant recipients (Grattan et al., 1989; McDonald et al., 1989; Koskinen et al., 1994). Also in the apparently immunocompetent host CMV has been implicated in the genesis of atherosclerosis (Melnick et al., 1990, 1994, 1995; Nieto et al., 1996), in coronary restenosis after angioplasty (Speir et al., 1994; Zhou et al., 1996) and in inflammatory aortic diseases (Tanaka et al., 1994; Yonemitsu et al., 1996).

The presence of CMV in the vessel wall, especially in the atherosclerotic vessel wall suggests that the virus could be involved in the genesis of atherosclerosis (Hendrix et al., 1990). Recent data of experiments in animal models experiments support this role.

The purpose of this paper is to give an overview of the knowledge about CMV and its

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PII: S0166-3542(99)00051-0

role in vascular pathology; especially the contribution of CMV in the pathogenesis of atherosclerosis, restenosis and transplant associated atherosclerosis will be discussed.

In addition, some important aspects of the interaction of the virus with cells of the vascular wall and some mediators involved in atherogenesis will be reviewed.

2. Atherogenesis

2.1. Atherosclerosis

Atherosclerosis is the principal underlying cause of coronary heart disease which is the most common death in industrialized countries.

The atherosclerotic process starts as a protective response to injury of the wall of the artery. It progresses to the formation of atherosclerotic plaques which narrow and at the end may obstruct the lumen of the affected artery. If plaque rupture occurs platelet aggregation occurs thereby creating a thrombus.

Atherosclerosis is the common background behind processes such as: acute myocardial infarction, unstable angina and ischemic stroke.

It is now generally accepted that atherosclerosis develops as a response-to-injury (Ross, 1993). Various insults are thought to be important such as hyperlipidemia, cigarette smoking but probably also infections.

The first and perhaps the most important step in the development of the lesion is the injury or dysfunction of endothelial cells. This is followed by accumulation of leukocytes (monocytes, macrophages and lymphocytes) in the intima.

In a hypercholesterolemic environment the macrophages ingest oxidised lipid and by this way become foam cells. Under the influence of various growth factors the smooth muscle cells proliferate and secrete extracellular matrix leading to an intimal thickening.

During the whole process several (soluble) factors and cells play an active role in recruitment, migration and proliferation of cells and synthesis of proteins such as extracellular matrix proteins and lipids.

2.2. Role of infectious agents in atherogenesis

The inability of well-known risk factors such as hypercholesterolemia and hypertension to completely explain the incidence of cardiovascular diseases has resulted in further research for new risk factors. Among them infections have gained an important topic of interest during the last years. Especially, *Chlamydia pneumoniae* and CMV have been studied in several centres.

In 1973, Benditt and Benditt reported that cells in atherosclerotic plaques had a monoclonal origin. They concluded that factors e.g. viruses could be involved in this process (Benditt and Benditt, 1973). In 1978 Fabricant reported that arterial lesions were found in chickens infected with a herpesvirus (Marek disease virus) (Fabricant et al., 1978). The lesions observed resembled those observed in human atherosclerosis. Further research showed that the process of atherosclerosis found in chickens infected with Marek's disease virus was preventable when the chickens were immunized with herpesvirus of Turkey (HVT) (Fabricant, 1985). This result further proved that MDV may play an etiological role in atherosclerosis.

The atherosclerotic lesions found in their chickens developed both with a normo-cholesterolemic and a cholesterol-rich diet. A cholesterol diet, however, changed the lesions from proliferative to fatty-proliferative. The virus could be found in the arterial walls by immunofluorescence techniques (Minick et al., 1979).

Also in humans an association between herpesvirus infections and atherogenesis has been studied. Especially CMV is the virus from which potential relationship to atherosclerosis has been investigated. An association between CMV infection and atherosclerosis was originally based on case-control study of cardiovascular patients undergoing surgery (Adam et al., 1987).

In this study the incidence of positive CMV antibodies was higher in the surgical group than in the control group. Similar results were found comparing CMV, HSV-1 and HSV-2 antibody titers and the thickness of carotid arteries in patients with symptomatic carotid artery thickening (Sorlie et al., 1994).

2.3. CMV and atherosclerosis

During the last 5 years several investigators have reported observations implicating certain microorganisms in human atherosclerotic disease. Among them CMV has been presented as a possible primary etiologic factor or as a co-factor in the pathogenesis of atherosclerosis.

Two lines of evidence have been presented: firstly epidemiological evidence based on serological data indicating an association between antibodies and atherosclerotic disease, secondly the detection of the virus in the atherosclerotic lesions.

Epidemiological evidence of a potential role of CMV in the pathogenesis atherosclerosis has been provided by Adam et al. (1987). More recently, Nieto presented the results of a cohort study of CMV infection as a risk factor for carotid intimal-medial thickening (Nieto et al., 1996). In this study longitudinal data were obtained using a case-control methodology nested within a historical cohort. Antibody titers for CMV and HSV were measured in sera obtained in 1974 and the carotid intimal-medial thickening (IMT) was measured 13–18 years later. The subjects with high IMTs had higher mean CMV antibody titers in 1974 than the control subjects suggesting a relationship between atherosclerosis and CMV.

Although some epidemiological associations have been found, others did find such an association (Adler et al., 1998; Ridker et al., 1998). As has been concluded recently by Danesh and coworkers better and larger sero-epidemiological studies are needed to resolve these uncertainties (Danesh et al., 1997).

Detection of the virus (viral antigen or viral genome) in atherosclerotic vessel wall has been described by several groups starting by Melnick in the early eighties (Melnick et al., 1983). Later on this has been supported by work of others (Yamashiroya et al., 1988; Hendrix et al., 1989; Chiu et al., 1997).

In 1990 Hendrix and coworkers found that the amount of virus is correlated with the severity of the atherosclerotic process in the arterial tree (Hendrix et al., 1990). In this study CMV nucleic acids were demonstrated by PCR in 90% of samples taken from the abdominal aorta or the femoral artery of CMV-seropositive patients with severe atherosclerosis (grade III) undergoing vascular surgery, as compared with 53% of samples taken at autopsy from patients with low grade (grade I) atherosclerosis.

Although these studies suggest an etiologic role of the virus, the final proof is nevertheless incomplete. Since atherosclerosis is a multifactorial process, presence of the virus even in atherosclerotic material does not automatically indicate that the virus is a (co)factor in the pathologic process. For this purpose in vivo experiments in animals are very important since they make it possible to study the role of infection in the development of atherosclerosis in well defined conditions.

Infection experiments in mice have shown that especially in immunocompromised animals arteritic lesions develop. These inflammatory lesions are characterized by mononuclear cell infiltrates in the media and the adventitia. Total arterial lipid accretion was greater in the CMV infected mice than in the uninfected mice (Dangler et al., 1995; Berencsi et al., 1998). Presence of CMV antigens in arterial cells was dependent on the immune competence of the host (Presti et al., 1998).

These data indicate that as has been demonstrated in humans, CMV is able to infect vascular wall cells and to induce an inflammatory reaction within the vascular wall.

The contribution of CMV to the pathogenesis of atherosclerosis can be studied in mice developing atherosclerosis, e.g. apolipoprotein E (apoE)-knock out mice (Zhang et al., 1992). Preliminary data of such infection experiments in our laboratory have shown that indeed CMV infection leads to enhanced development of atherosclerotic lesions characterized by an increased size of early atherosclerotic lesions in the vessel wall (Nelissen-Vrancken et al., 1999).

2.4. Role of CMV in restenosis

The processes leading to restenosis and atherosclerosis are similar but are not identical. Atherosclerosis is a chronic ongoing process characterized by presence of foam cells, cholesterol

deposition, T-lymphocytes and macrophages suggesting an inflammatory process probably induced by multiple episodes of different forms of vascular injury. The process starts as a fatty streak and develops over a period of several years into an atherosclerotic plaque with lipid deposition and calcification.

Restenosis, in contrast, is triggered by an acute mechanical injury and leads to luminal narrowing within a period of weeks or months.

Although there are a lot of differences between both processes some of the mechanisms involved in restenosis are similar to those involved in atherogenesis.

For example the angioplasty-induced injury leads to the expression of growth factors, cytokines and adhesion molecules and activation of genes involved in inflammatory and immune responses. That CMV can play a role in the restenosis process has been described by the group of Epstein (Speir et al., 1994; Epstein et al., 1996).

The effect of CMV on restenosis was due to an interaction between the immediate early (IE) gene product of CMV, IE2-84, and p53 leading to inhibition of its transactivational activity.

The interaction of CMV IE proteins present in the vessel wall with p53 could have important implications for the process of restenosis. Firstly, CMV can inhibit the capacity of p53 to cause cell cycle arrest resulting in excessive SMC proliferation leading to formation of an neointima formation and thus to severe lumen narrowing. Secondly p53 can inhibit CMV induced apoptosis by interaction of IE2-84 (Zhu et al., 1995), with p53 and by this way contributes to an accumulation of SMC in the vessel wall.

Experiments in rats infected with rat CMV (RCMV) using the balloon denudation model support these findings in humans (Zhou et al., 1995).

2.5. CMV and transplant arteriosclerosis

After organ transplantation arteriosclerosis develops. The most characteristic manifestation is an ongoing inflammatory process resulting in a neointima formation in all arteries and arterioles of the graft. In contrast with classical atherosclerosis in which the process occurs focal and asymmetric, in transplant arteriosclerosis the manifestations are generalized and the intimal thickening is concentric. The process is characterized by a persistent perivascular and interstitional inflammation leading finally into concentric allograft arteriosclerosis (Hayry et al., 1993). In the pathogenesis of transplant arteriosclerosis immunologic factors due to histo- incompatibility between donor and recipient are essential keys in the process since in syngeneic grafts no or nearly no arteriosclerosis develops.

Several factors such as hyperlipidemia and prolonged ischemia enhance this effect.

In 1989, a correlation between CMV infection and development of transplant arteriosclerosis was found in cardiac transplant recipients (Grattan et al., 1989).

Transplant arteriosclerosis was more frequently detected in recipients with CMV infection than in uninfected patients. The rate of graft loss was also significantly higher in CMV infected patients than in controls. Similar observations were done by others (McDonald et al., 1989; Loebe et al., 1990). Also the presence of CMV nucleic acids could be detected in coronary arteries of cardiac transplant recipients with severe vasculopathy (Hruban et al., 1990).

The effect of CMV infection on the development of transplant arteriosclerosis was studied in detail by Lemström and coworkers using the elegant rat aortic transplant model (Lemstrom et al., 1993, 1994a,c). By using this model it was clearly shown that CMV infection leads to enhanced perivascular inflammatory processes starting one week after transplantation and persisting during several months. Especially monocytes and T-lymphocytes are involved in the inflammation. Neointimal formation reached high levels at 3–6 months post transplantation.

For the development of transplant arteriosclerosis histoincompatibility between donor and recipient are necessary (Bruning et al., 1994; Lemstrom et al., 1994b; Li et al., 1998). The enhanced effect of CMV infection on the process was prevented when the animals were treated with either ganciclovir or cidofovir (Jacobson, 1997; Safrin et al., 1997).

3. Interaction between cmv infection and the vessel wall

3.1. CMV infection and endothelial cells

In the 'response-to-injury' hypothesis of atherosclerosis damage to the endothelium is an essential step in the early process that proceeds plaque formation (Ross, 1993).

Endothelial cells form a barrier between circulating blood and the vessel wall. The endothelium is not an inert layer but is involved in maintaining the procoagulant/anticoagulant balance in the blood, in angiogenesis, in wound healing and in inflammation. It is also the source of diverse mediators which modulate the contraction and relaxation of vascular smooth muscle cells.

In normal conditions the endothelium perform a variety of roles: it provides a non-thrombotic surface, it inhibits smooth muscle cell proliferation, it prevents adhesion of blood cells. Dysfunction of endothelium disturbs its function and contributes to atherosclerosis.

Productive infection of endothelial cells by CMV is rather limited and is probably restricted to microvascular endothelial cells (Myerson et al., 1984; Lee, 1989; Wilcox et al., 1990; Persoons et al., 1998).

In large vessels hardly any infected endothelial cells are found (Span et al., 1992; Li et al., 1996), although 'activation' of these cells is found in the infected host (Span et al., 1992; Span et al. 1993). The interaction of CMV with endothelial cells is rather complex and our knowledge is rather limited. Several factors are important in this interaction, such as the virus strain, the activation state of the cells, and the origin of the endothelial cells (Waldman et al., 1989; Scholz et al., 1996; Vossen et al., 1996; Slobbe van Drunen et al., 1997; Vossen et al., 1997).

The observation that in the in vivo situation no virus containing endothelial cells are found does not implicate that the interaction between CMV and endothelium has no part in atherosclerosis. CMV may play a role in this process either directly via contact with or entry in these cells or

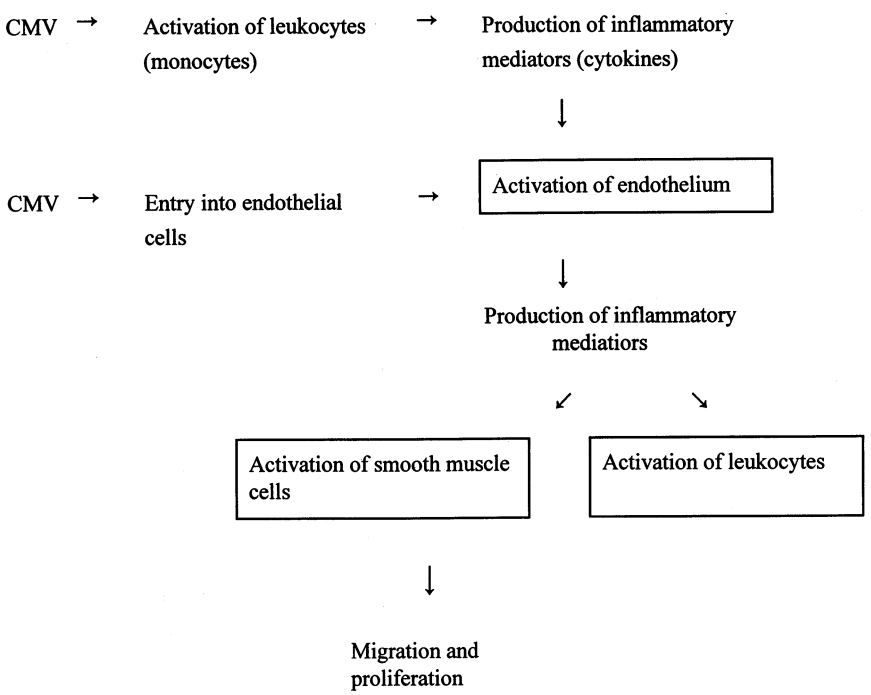

Fig. 1. Virus induced activation of endothelial cells.

indirectly via inflammatory mediators secreted (Fig. 1).

CMV infection can induce increased cytokine production such as interleukin (IL) -1, IL-6 and IL-8. In addition interferon (IFN)α, β and γ and tumor necrosis factor (TNF)-α are upregulated (Woodroffe et al., 1993; Almeida et al., 1994; Craigen and Grundy, 1996; Murayama et al., 1998). This upregulation of cytokine production can be due to infection of the cell itself (direct effect) or to its interaction with other cells such as leukocytes interacting with the infected cells.

Leukocyte activation, a phenomenon occurring during infections, is associated with release of substances that are capable of mediating vascular damage. Again cytokines such as IL-1, TNF-α and IFN are important (Grundy, 1998). These substances can promote adherence of leucocytes to the endothelium via the expression of adhesion molecules, such as VCAM-1, ICAM-1, LFA-3 (Span et al., 1991; Almeida et al., 1994; Craigen and Grundy, 1996).

Another important effect of infection of endothelial cells is the potential of viruses to alter endothelial cells from a anticoagulant phenotype to a procoagulant state (van Dam-Mieras et al., 1992; van Geelen et al., 1995). These prothrombic effects of infection on the endothelial cells have been detected for several herpesviruses (Nicholson and Hajjar, 1998). Recently interesting data in this field were described by Nieto and coworkers (Nieto et al., 1997). In this study an association between CMV infection and chronic hypercoagulability was found. CMV antibody titers were associated with plasma levels of von Willebrand factor, factor VIII and protein C, suggesting that also in the in vivo situation CMV infection has effects on thrombosis and coagulation.

In vivo experiments in CMV infected rats further support these in vitro data (Span et al., 1992, 1993). In these experiments CMV infection leads to activation of endothelial cells and to increased adhesion of leucocytes to the endothelium.

3.2. CMV infection and migration and proliferation of smooth muscle cells

Although inflammation provides the mean pathophysiologic basis for the development of atherosclerosis, also the migration and proliferation of smooth muscle cells is an important phenomenon in the process. Chemotactic factors (for migration) and growth factors (for proliferation) are involved.

From in vitro data it is known that CMV is able to replicate in smooth muscle cells (Tumilowicz et al., 1985), whereas in vivo data are less clear. During acute infection presence of infectious virus or viral antigens in smooth muscle cells is rather limited. Infection of small animals (mice and rats) reveals that in the immunocompetent host the vascular wall does contain low concentrations of infectious virus during the acute phase of the infection, i.e. during the first weeks after inoculation. Later on, no infectious virus is detectable in the vascular wall, while viral DNA persist for longer periods (Blok et al., to be published). After balloon injury, however, massive active CMV infection of neointimal smooth muscle cells occurs (Persoons et al., 1994). Further experiments, using the same model, have shown that the phenotype of the smooth muscle cells is a crucial factor in the permissiveness to CMV infection (Persoons et al., 1997, 1999). Although the infection of smooth muscle cells during the acute phase of the infection is rather limited and depends largely on the immune competence of the host, several studies have shown that the smooth muscle cell layer is a site of latency (Hendrix et al., 1989, 1991; Melnick et al., 1994). This suggest an important role of smooth muscle cells in the process of atherogenesis. Especially, when reactivation of the virus occurs, smooth muscle cells may play an important role in the process of atherogenesis by induction of local cytopathic effects and inflammatory responses.

Since the number of CMV DNA containing specimens was significantly higher in the atherosclerotic vessel wall than in the controls it is tempting to speculate that the presence of the virus is involved in the atherosclerotic process (Hendrix et al., 1990).

One possibility is that the presence of the virus in these cells triggers the cytokine pathway leading to upregulation of growth factors and of

chemokines. Evidence that CMV infection in vivo leads to cell proliferation has been shown in rat studies (Lemstrom et al., 1993). By using the rat aortic allograft transplant model it was shown that CMV enhances the proliferation of smooth muscle cells leading to an enhanced neointima formation. Although the mechanism involved in the latter process is not clear, increased expression of growth factors such as platelet derived growth factor (PDGF)-BB mRNA and basic fibroblast growth factor (B-FDF) mRNA were found (Lemstrom et al., 1994a).

Another possibility is that chemokine receptors encoded by the viral genome are involved in the migration of cells in the vascular wall.

The genomes of several members of the herpesvirus family contain sequences encoding protein with homology to G-protein-coupled receptors for chemotactic cytokines (Gao and Murphy, 1994; Gompels et al., 1995). Although the role of chemokines and virally encoded chemokine receptors in CMV pathogenesis are unknown the presence of putative chemokine receptors suggest a role in the interaction of virusinfected cells with inflammatory processes via chemokines or homologs (Beisser et al., 1998).

3.3. CMV and lipid metabolism of vascular cells

Herpesvirus infections have effects on cellular cholesterol accumulation (Fabricant et al., 1973). Cultured chicken aortic smooth muscle cells infected with Marek disease virus were shown to contain higher amounts of cholesterol and other lipids than noninfected cells (Fabricant et al., 1981b).

In 1987, Hajjar and coworkers demonstrated that HSV infection of smooth muscle cells leads to decreased lysosomal and cytoplasmic cholesterol ester hydrolytic activity (Hajjar et al., 1986). More recently, it has been shown that CMV increases uptake of oxidized LDL an effect mediated by CMV induced expression of class A scavenger receptor. Since a similar effect was seen when rat smooth muscle cells were infected with human CMV (leading to abortive infection), it was concluded that only the IE genes of CMV are necessary for this effect (Zhou et al., 1996).

Table 1
Phenomena supporting the hypothesis that CMV plays a role in atherogenesis

1	CMV causes systemic infection leading to infection of endothelial cells and smooth muscle cells.
2	CMV induces loss of anticoagulant properties of vascular endothelial cells and acquisition of procoagulant properties when infected in vitro.
3	Entry of CMV into endothelial cells activates these cells resulting in the production of cytokines and chemotactic factors leading to attraction and adherence of leucocytes to the endothelium.
4	CMV infection leads to an inflammatory response where monocytes and T-lymphocytes are involved.
5	CMV can persists in smooth muscle cells and can stimulate the proliferation of these cells via interaction with p53.
6	CMV disturbs the lipid metabolism of vascular cells.
7	Infection of animals leads to induction of atherosclerotic processes characterized by a local inflammatory reaction within the vessel wall and proliferation of smooth muscle cells.
8	An epidemiological correlation exists between the presence of CMV antibodies and atherosclerosis and restenosis.
9	CMV infection has an enhancing effect on transplant associated arteriosclerosis. This effect can be prevented by effective treatment with antiviral drugs

Also in vivo effects of CMV infection on the lipid metabolism were detected (Berencsi et al., 1998). In this study the percentage of LDL-cholesterol, the major contributor to atherosclerotic plaques, was significantly increased in CMV infected mice.

4. Conclusion

The results of all these studies are far from the final word. The evidence that infections play an important role is delivered from several studies (summarized in Table 1) but remain largely circumstantial.

One general accepted hypothesis is that inflammation triggered by an infectious micro-organism might be a keyfactor in atherogenesis.

Since CMV has been detected in the vessel and even more in the atherosclerotic vessel wall than in the unaffected vessel wall, its tempting to speculate that persistent virus contributes to the ongo-

ing process of atherogenesis. More animal studies are needed to provide evidence that CMV might contribute to plaque formation Table 1.

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