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CMV-induced class II antigen expression in various rat organs

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Abstract Cytomegalovirus (CMV) is thought to trigger acute or chronic allograft rejection by inducing the expression of MHC class II antigens in the graft. This induction may be mediated by γ-interferon or directly by CMV. In this study, we have investigated which structures in the rat kidney, liver, and heart are responsive to CMV-induced class II expression in vivo. Rats were infected with rat CMV, the organs were harvested during the acute phase of infection, and the virus was demonstrated by culture from each organ. Direct CMV antigen detection was performed on frozen sections to demonstrate the detailed localization of CMV in the organs. In the kidney, CMV antigens were found in the vascular endothelium, in tubular cells, and scattered in the glomeruli. In the liver, the vascular structures and parenchyma contained CMV antigens. In the heart, CMV antigens were seen only in the capillary endothelium. Class II antigen expression was demonstrated by a monoclonal antibody and immunoperoxidase techniques. The induction of class II molecules was recorded in exactly the same cellular structures as those in which CMV antigens were detected.

Key words CMV, rat, organ transplantation · Class II expression, CMV, rat

Introduction

Cytomegalovirus (CMV) infection is a major complication after organ transplantation [22]. In addition to a variety of clinical manifestations, an association between acute or chronic rejection and CMV infection has been reported in several series of renal [16, 23, 29], liver [1, 20], and heart [10, 15, 19, 28] transplants. An upregulation of MHC antigens – MHC class II antigen expression – in the graft has been demonstrated at least in kidney and liver allografts during CMV infection [13, 29]. It has been suggested that this upregulation of class II antigens is mediated by γ-interferon that is produced by activated lymphocytes during the viral infection [29].

The effect of γ-interferon at the molecular level, namely, the attachment of γ-interferon to its receptor on the cell surface, is necessary as a second signal in the induction of class II antigen expression [8]. However, a direct viral induction of class II molecules has been described for different cell types [12, 17, 18]. We have previously demonstrated CMV-induced class II expression in rat heart endothelial cells in the absence of interferon-γ [26, 27], which suggests that a direct mechanism of upregulation of class II expression by the CMV might exist, at least in vitro. This induction of class II antigen expression seems to be γ-interferon-independent and caused by the virus itself. A direct induction of class II expression by CMV may also play a role in various organs in vivo.

In this study, we have investigated the upregulation of MHC class II antigens associated with rat CMV infection in various structures of the rat kidney, liver, and heart in vivo.

Materials and methods

Rat CMV infection

Inbred DA rats were used. The rats were infected by intraperitoneal (i.p.) injection of 10^5 plaque-forming units (PFU) of rat cytomegalovirus (RCMV) strain (RB61), as described previously [4]. After 6–7 days, when the infection was in its acute phase [5], the animals were sacrificed and the organs harvested. Altogether, five control animals and five infected animals were studied.

Viral cultures

For viral cultures, the tissues were disaggregated and suspended in tissue culture medium. Rat embryonic fibroblasts and standard virus culture conditions were used. Both conventional cultures and rapid shell vial cultures [25] were performed, and the virus was demonstrated in the cultures by indirect immunofluorescence and a monoclonal antibody against RCMV-specific early nuclear proteins and late antigens (mixed antibody) as described elsewhere [6]).

Direct demonstration of RCMV antigens in various tissues

Frozen sections of rat heart, kidney, and liver tissues were fixed in acetone, and the presence of the virus in various organs was demonstrated by indirect immunofluorescence and the RCMV-specific monoclonal antibody. Normal rat organs were used as controls.

Table 1 Detection of CMV and class II antigens in different rat organs. The intensity of antigen expression ranged from – to +++ (– none, + weak, ++ moderate, +++ strong staining)

Organ	CMV antigens	Class II antigens	
		During CMV Infection	Normal animals
Kidney			
Tubuli	++/+++[a]	+/+++[a]	–
Glomeruli	+++[b]	+++[b]	–
Interstitial cells	++/+++	++/+++	+
Endothelial cells	++/+++	++/+++	+
Liver			
Parenchyma			
Hepatocytes	++/+++[c]	+/+++[c]	–
Endothelial cells	+++	++/+++	+
Portal area			
Bile ducts	++/+++	++/+++	–
Endothelial cells	+++	++/+++	+
Sinusoids			
Kupffer cells	+++[d]	++/+++[d]	+++
Endothelial cells	+++	++	+
Heart			
Myocardial cells	–	–	–
Interstitial cells	++[e]	+/++[e]	+
Endothelial cells	+/++[f]	+/++[f]	–

[a] Antigens were not found in all tubuli
[b] Strong, scattered, positive staining
[c] Diffuse staining
[b] Number of cells was increased
[e] Scattered, positive staining
[f] Only a few scattered positive cells in the capillaries

Demonstration of MHC class II antigens

An indirect immunoperoxidase technique and a monoclonal antibody against rat MHC class II (MAS 043c, Seralab, Sussex, England) were used. Normal mouse IgG antibody was employed as a negative control. The frozen sections were first incubated with the monoclonal mouse antibody. A peroxidase-conjugated rabbit anti-mouse antibody (Dako, Copenhagen, Denmark) and a peroxidase-conjugated goat anti-rabbit antibody (Tago Inc. Burlingame, Calif., USA) were used as second and third antibodies, respectively. The reaction was revealed by AEC (3-amino-9-ethyl carbazole) solution containing hydrogen peroxide. Mayer's hemalum was used for counterstaining.

Demonstration of T lymphocytes

T lymphocytes were identified using the immunoperoxidase technique mentioned above. Monoclonal antibodies against T-helper cells (MAS 1131c, Seralab) and T-suppressor/cytotoxic cells (MAS 041c, Seralab) were used. Activated T cells were identified by a monoclonal antibody against IL-2 receptor (a gift from Dr. J. Kupiec-Weglinski, Harvard Medical School, Boston, Mass., USA).

Results

RCMV infection in various organs

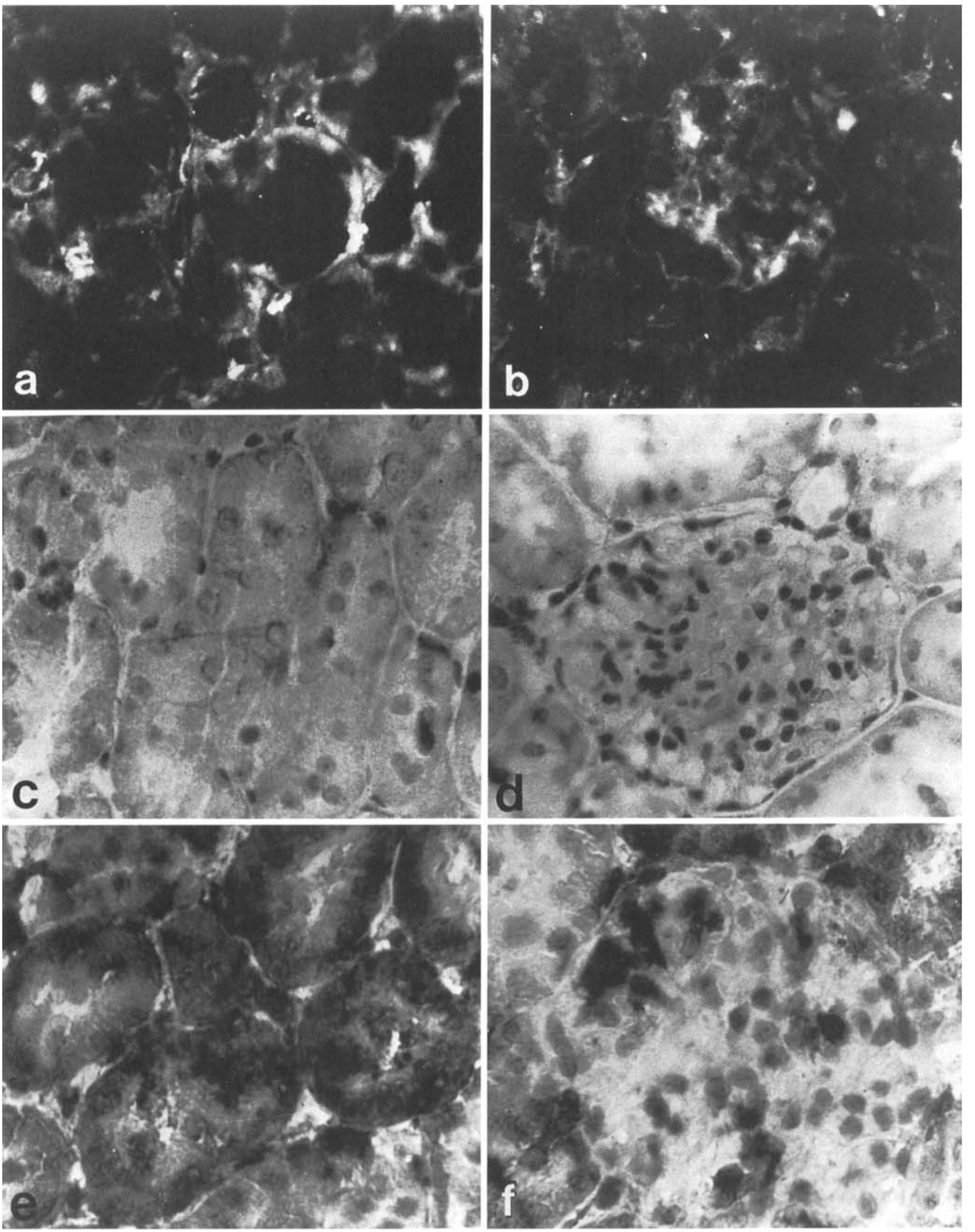
Using viral culture techniques, RCMV could be detected from both kidney and liver tissues but not from heart tissues. The more detailed analysis, using direct detection of RCMV antigens in the frozen organ section, demonstrated the location of the infection in each of the organs (Table 1).

Kidney

RCMV antigens were detected in interstitial macrophage-like cells, in most vascular endothelial cells, and in tubular cells. However, not all tubuli seemed to be infected, although some areas were rather strongly infected (Fig. 1 a). In glomeruli, only a few scattered cells (mostly mesangial cells) were positive for the virus (Fig. 1 b).

Liver

In the portal area, the vascular endothelial cells of the biliary artery and vein, as well as the epithelial cells of the bile ducts, were often RCMV-positive. In the parenchyma, large areas of hepatocytes expressed RCMV antigens (Fig. 2 a), as well as sinusoidal and central vein endothelial cells. Also, several Kupffer cells were infected.


a
b
c
d
e
f

Heart

Only very few RCMV-infected cells were found in the heart tissue. All myocardial cells were negative for the virus, but a few scattered CMV antigen-positive endothelial cells were found in the capillaries (Fig. 3 a).

The organs of uninfected animals were used as controls, and no positive staining for RCMV antigens could be seen. The histology of the organs was normal in the infected animals, no viral inclusions were observed, and no tissue damage was caused by the virus.

MHC class II expression in various organs

In normal animals, the expression of class II antigens was faint and seen in vascular endothelia of the kidney and liver but not of the heart. Also, in the normal liver, the Kupffer cells were class II-expressing. All epithelial cells of the kidney and liver, as well as myocardial cells, were completely negative for class II expression. In CMV-infected animals, a strong upregulation of class II antigens was recorded in kidney and liver parenchyma, as well as in vascular structures. In the heart, the upregulation was recorded in vascular endothelial cells only. The intensity and location of class II antigen staining correlated with that of CMV expression. The location of induced class II expression in each organ is described in detail and summarized in Table 1.

Kidney

Intensive staining with anti-class II antibody was seen in the tubular epithelium (Fig. 1 e), especially in the areas of CMV-infected tubuli. In addition, the vascular endothelium of capillaries and larger vessels strongly expressed class II antigens. In the glomeruli, which in normal animals were absolutely negative (Fig. 1 d), a strong, scattered, positive staining was recorded in CMV-infected animals (Fig. 1 f). Most of the stained cells were mesangial cells.

Liver

The hepatocytes throughout the parenchyma showed moderate to intense diffuse staining with anti-class II antibody. The Kupffer cells, scattered in the sinusoids, were strongly stained and the sinusoid endothelium (Fig. 2 c) moderately stained. In the portal area, both bile

Fig. 1 a–f CMV antigens were demonstrated by immunofluorescence staining both in **a** tubuli and **b** glomeruli during the infection. In normal kidneys, **c, d** both structures were class II-negative, but during CMV infection, **e** tubuli and **f** partly glomeruli became strongly class II-positive, as demonstrated by immunoperoxidase staining

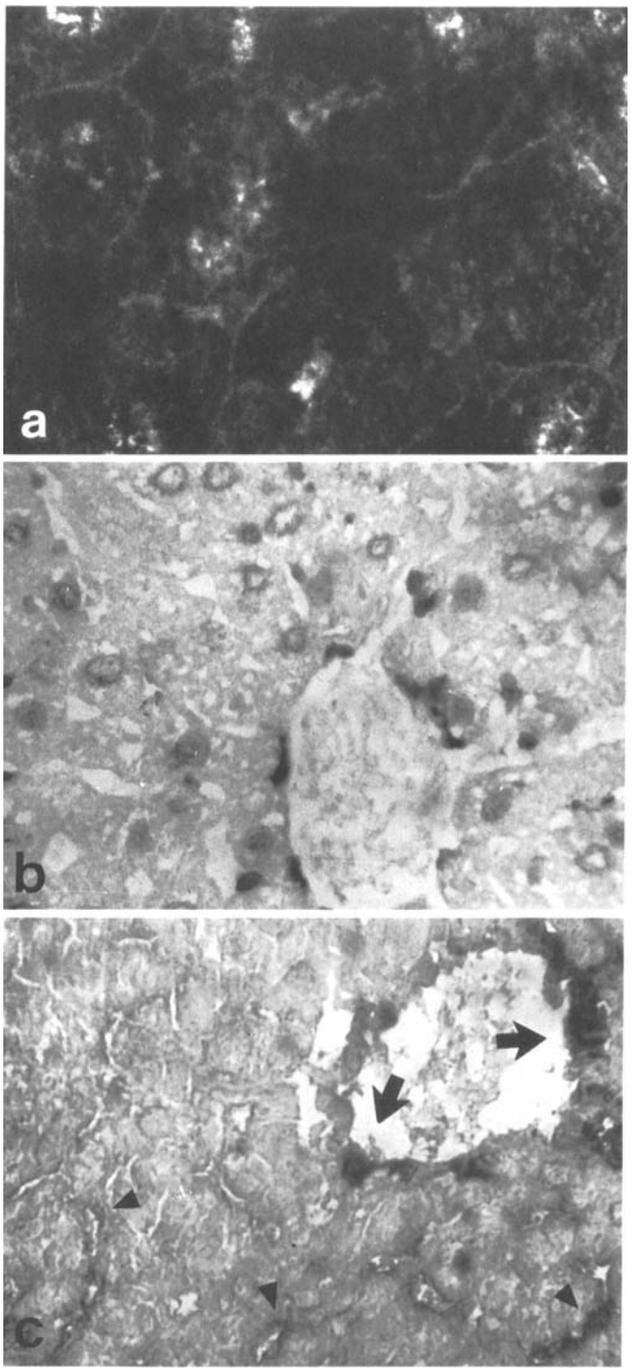


Fig. 2 **a** CMV antigens were demonstrated in hepatocytes during the infection. **b** Class II expression was not seen in normal specimens, **c** but during CMV infection, a clear positive reaction was demonstrated in hepatocytes (diffuse staining) and in endothelial cells of central veins *(arrows)* and sinusoids *(arrowheads)*

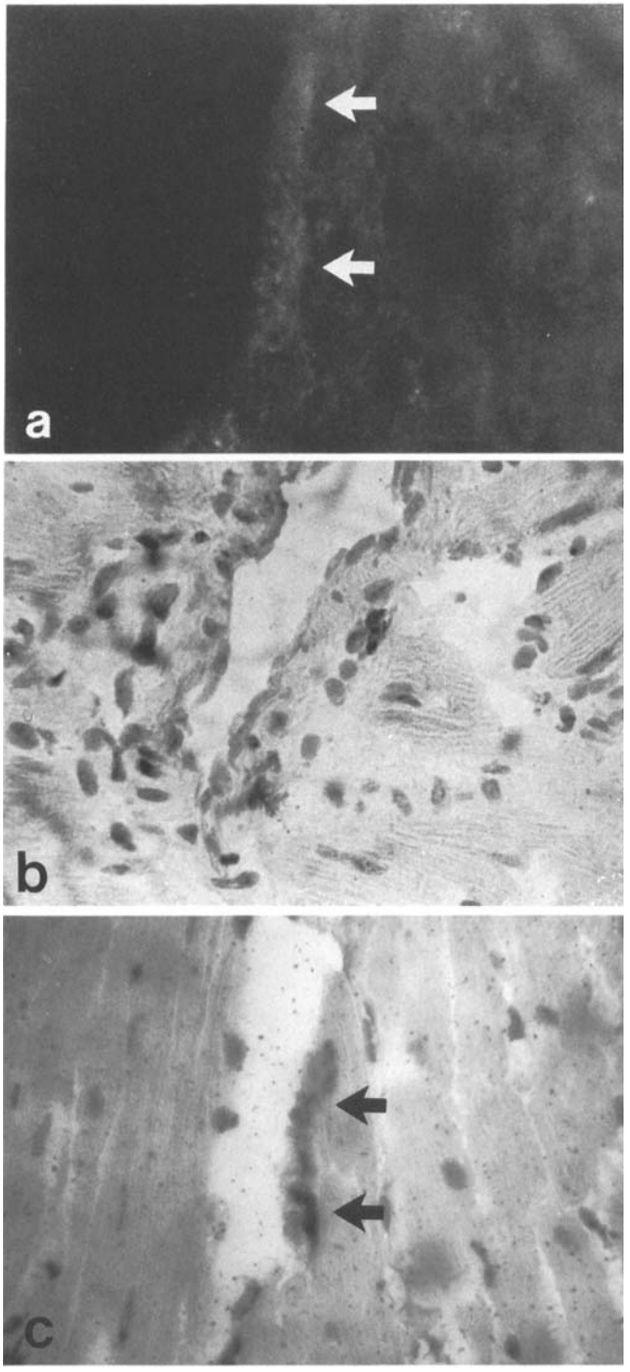


Fig. 3 **a** CMV antigens were found only in a few endothelial cells of vascular structures of the rat heart during the infection *(arrows)*. **b** No class II antigens were detectable in endothelial cells in normal hearts, **c** but a few scattered vascular endothelial cells expressed class II antigens during CMV infection *(arrows)*

ducts and the vascular endothelium of arteries and veins were strongly positive. Also, the endothelial cells of the central vein (Fig. 2c) were all positive.

Heart

In general, the heart tissue of the infected animals was not as rich in class II antigens as the tissues of the kidney and liver. The myocardial cells were absolutely negative for class II expression. However, the endothelial cells of the capillaries showed a clear reaction to anti-class II antibody (Fig. 3c), which was not recorded in normal animals (Fig. 3b). The larger vascular structures remained negative for class II expression.

In these organs, neither infiltration of inflammatory cells nor IL-2 receptor-positive T lymphocytes were detected.

Discussion

These results support the suggestion that CMV induces the expression of class II antigens in parenchymal structures of several organs. An intense increase in class II molecules on the surface of most endothelial cells was recorded. Also, in the epithelial cells of kidney tubuli as well as in glomeruli, class II induction appeared. Liver parenchymal cells, hepatocytes, and bile duct epithelial cells also became strongly positive for class II antigen expression. However, myocardial cells remained class II-negative, and the expression was recorded in the vascular endothelium only. CMV antigens were detected in exactly the same structures as those in which the induction of class II molecules was seen. The histology of the organs remained normal during the viral infection and no morphological changes or IL-2 receptor-positive, activated T lymphocytes were seen.

In kidney transplantation, an association has been recorded between acute allograft rejection, parenchymal cell class II expression, and CMV infection [29]. In liver allografts too hepatocytes and other parenchymal cells in the graft demonstrate a strong class II expression during CMV infection [13]. Although there is no causal association with acute liver rejection, clear evidence of an association with chronic rejection exists [1, 20]. On the other hand, some controversial results have also been published [21]. CMV DNA has also been detected in the hepatocytes of livers exhibiting chronic rejection [1]. An especially significant association between CMV and chronic rejection has been reported after heart transplantation [10, 28]. Chronic heart allograft rejection manifests itself as accelerated arteriosclerosis and affects the vascular structures. CMV nucleic acids have been found in the vascular walls of arteriosclerotic patients too [11].

It has been suggested that the increased expression of class II antigens in the graft during CMV infection triggers rejection [29]. This is thought to be mediated by γ-interferon that is produced by activated T cells during viral infection. Our rat model demonstrates that CMV infects the kidney and liver parenchyma and that class II induction occurs in the infected cells without lymphocyte infiltration in the organ. In the heart, the only target of CMV was the vascular endothelium, in which class II expression was also induced. One might assume that this induction occurs in the infected cells without the help of infiltrated T cells and γ-interferon. However, T-cell activation may occur in the circulation, and the induction of class II expression may be caused by a systemic effect of γ-interferon. Alternative pathways that induce class II expression may also exist via different cytokines or via a combination of them.

In nonimmunosuppressed, CMV-infected rat heart allograft, the inflammatory episode occurred during the 1st week after transplantation when the recipient rats were inoculated with CMV on the 1st post-transplant day. In uninfected rat allografts, the inflammatory peak occurred only 1 month after infection [14]. In addition, most endothelial cells were class II-positive in capillaries of CMV-infected grafts. In uninfected grafts, the expression of class II antigens was lower (K. Lemström, personal communication).

In CMV-infected rat heart allografts triple-immunosuppressed with CyA (20 mg/kg per day), methylprednisolone (0.5 mg/kg per day), and azathioprine (2 mg/kg per day), an inflammatory episode and increased MHC class II expression have been demonstrated during the 1st week after transplantation in the perivascular space of capillaries and arterioles (K. Lemström, personal communication). Endothelial cells were class II-positive mainly in capillaries, and the number of positive cells was higher in CMV-infected grafts than in uninfected grafts. On the other hand, class II expression was lower than in nonimmunosuppressed rats.

The direct effect of CMV on class II molecules is most likely based on the virus' own molecules, which modify the host's genome products during infection. During inflammatory infiltration of an allograft, CMV activates γ-interferon production of T cells and further stimulates MHC class II molecule production.

In previous studies, direct induction of MHC class I – but not class II – antigens by CMV has been demonstrated in human endothelial cell cultures [7, 24]. However, these experiments were done on human umbilical vein endothelial (HUVE) cells, and not on normal endothelial cells from vascular structures of organs. In our previous studies [26, 27], γ-interferon-independent CMV-induced class II expression was recorded in rat heart endothelial cells in culture. Also, direct virus-induced class II expression has been demonstrated in other types of cells [12, 17, 18], indicating that viral agents, during binding, penetration, or replication, can cause some γ-interferon-independent events that lead to class II expression in the target cells.

Recently, new information regarding the mechanisms by which CMV infection may contribute to graft rejection has been published. DNA sequence analyses have demonstrated not only that CMV encodes a molecule similar to the MHC class I antigen [3], but also that there is a sequence homology between some CMV-encoded molecules and the HLA-DR β chain [9] and α chain [2]. Immunological crossreactivity between CMV IE-2 antigen and DR antigen has also been recorded [9]. Thus, the immunoresponse generated against CMV could also react with class II antigens, or vice versa.

Taken together, we have demonstrated which cellular structures in various organs are targets for CMV in vivo. The induction of class II expression has been studied in the situation where CMV has not attracted inflammatory cells. Class II expression appears in CMV-infected cells and may even be caused by the virus itself. However, whether this is "real" class II expression or a CMV-encoded gene product that mimics class II expression is not clear.

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References

1. Arnold JC, Portmann BC, O'Grady JG, Naoumov NV, Alexander GJ, Williams R (1992) Cytomegalovirus infection persists in the liver graft in the vanishing bile duct syndrome. Hepatology 16: 285–292
2. Baum H, Butler P, Davies H, Sternberg MJE, Burroughs AK (1993) Autoimmune disease and molecular mimicry: an hypothesis. Trends Biochem Sci 18: 140–144
3. Beck S, Barrell BG (1988) Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. Nature 331: 269–272
4. Bruggeman CA, Debie WHM, Grauls G, Majoor G, Boven CPA van (1983) Infection of laboratory rats with a new CMV-like virus. Arch Virol 76: 189–199
5. Bruggeman CA, Meijer H, Bosman F, Boven CPA van (1985) Biology of RCMV infection. Intervirology 24: 1–9
6. Bruning JH, Debie WHM, Dormans PHJ, Meijer H, Bruggeman CA (1987) The development and characterization of monoclonal antibodies against rat cytomegalovirus induced antigens. Arch Virol 94: 55–70
7. Dorp WT van, Jonges E, Bruggeman CA, Daha MR, Es LA van, Woude FJ van der (1989) Direct induction of MHC class I, but not class II, expression on endothelial cells by cytomegalovirus infection. Transplantation 48: 469–472

8. Dower SK, Smith CA, Park LS (1990) Human cytokine receptors. J Clin Immunol 10: 289–299
9. Fujinami RS, Nelson JA, Walker L, Oldstone MBA (1988) Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DR β chain: a means for graft rejection and immunosuppression. J Virol 62: 100–105
10. Grattan MT, Moreno-Cabral CE, Starnes VA, Oyer PE, Stinson EB, Shumway NE (1989) Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. JAMA 261: 3561–3566
11. Hendrix MGR, Dormans PHJ, Kitslaar P, Bosman F, Bruggeman CA (1989) The presence of cytomegalovirus nucleic acids in arterial walls of atherosclerotic and nonatherosclerotic patients. Am J Pathol 5: 1151–1157
12. Kannagi M, Kiyotaki M, King NW, Lord CI, Letvin NL (1987) Simian immunodeficiency virus induces expression of class II major histocombatibility complex structures on infected target cells in vitro. J Virol 61: 1421–1426
13. Lautenschlager I, Höckerstedt K, Salmela K, Isoniemi H, Holmberg C, Jalanko H, Häyry P (1990) Fine needle aspiration biopsy in the monitoring of liver allografts: different cellular findings during rejection and CMV infection. Transplantation 50: 798–803
14. Lemström KB, Bruning JH, Bruggeman CA, Lautenschlager IT, Häyry P (1993) Cytomegalovirus infection enhances smooth muscle cell proliferation and intimal thickening of rat aortic allografts. J Clin Invest 92: 549–558
15. Loebe M, Schüler S, Zais O, Warnecke H, Fleck E, Hetzer R (1990) Role of cytomegalovirus infection in the development of coronary artery disease in the transplanted heart. J Heart Transplant 9: 707–711
16. Lopez C, Simmons RL, Mauer SM, Najarian JS, Good RA (1974) Association of renal allograft rejection with virus infections. Am J Med 56: 280–289
17. Massa PT, Dörries R, Meulen V ter (1986) Viral particles induce Ia antigen expression on astrocytes. Nature 320: 543–546
18. Massa PT, Schimpl A, Wecker E, Meulen V ter (1987) Tumor necrosis factor amplifies measles virus-mediated Ia induction on astrocytes. Proc Natl Acad Sci USA 84: 7242–7245
19. McDonald K, Rector TS, Braunlin EA, Kubo SH, Olivari MT (1989) Association of coronary artery disease in cardiac transplant recipients with cytomegalovirus infection. Am J Cardiol 64: 359–362
20. O'Grady JG, Sutherland S, Harvey F, Calne RY, Alexander GJM, Donaldson PT, Portmann B, Williams R (1988) Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bile duct syndrome after liver transplantation. Lancet II: 302–305
21. Paya CV, Wiesner RH, Hermans PE, Larson-Keller JJ, Ilstrup DM, Krom RA, Moore SB, Ludwig J, Smith TF (1992) Lack of association between cytomegalovirus infection, HLA matching and the vanishing bile duct syndrome after liver transplantation. Hepatology 16: 66–70
22. Rubin RH, Tolkoff-Rubin NE (1989) The problem of cytomegalovirus infection in transplantation. In: Morris PJ, Tilney NL (eds) Progress in transplantation. New York, Churchill Livingstone, pp 89–114
23. Rubin RH, Tolkoff-Rubin NE, Oliver D, Rota TR, Hamilton J, Betts RF, Pass RF, Hillis W, Szmuness W, Farrell ML, Hirsch MS (1985) Multicenter seroepidemiologic study of the impact of cytomegalovirus infection on renal transplantation. Transplantation 40: 243–249
24. Sedmak DD, Roberts WH, Stephens RE, Buesching WJ, Morgan LA, Davis DH, Waldman WJ (1990) Inability of cytomegalovirus infection of cultured endothelial cells to induce HLA class II antigen expression. Transplantation 49: 458–462
25. Smith TF (1987) Rapid methods for diagnosis of viral infections. Lab Med 18: 16–20
26. Ustinov JA, Loginov RJ, Bruggeman CA, Meide PH van der, Häyry PJ, Lautenschlager IT (1993) Cytomegalovirus induces class II expression in rat heart endothelial cells. J Heart Lung Transplant 12: 644–651
27. Ustinov J, Loginov R, Bruggeman C, Meide P van der, Häyry P, Lautenschlager I (1993) Direct induction of class II antigens by cytomegalovirus (CMV) in rat heart endothelial cells. Transplant Proc 25: 1143–1144
28. Weimar W, Balk AHMM, Metselaar HJ, Mochtar B, Rothbarth PH (1991) On the relation between cytomegalovirus infection and rejection after heart transplantation. Transplantation 52: 162–164
29. Willebrand E von, Petterson E, Ahonen J, Häyry P (1986) CMV infection, class II antigen expression, and human kidney allograft rejection. Transplantation 42: 364–367