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Cytomegalovirus infection of human kidney cells in vitro

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Cytomegalovirus infection of human kidney cells in vitro. To study which structures of a kidney allograft are the main targets for cytomegalovirus (CMV), human glomerular epithelial and mesangial cells, as well as tubular epithelial and endothelial cells were isolated by steel meshes of different pore sizes and enzymatic treatments. The various cultured cell types were characterized by morphology and specific antibodies. Human CMV was inoculated onto cell monolayers using two different culture methods: conventional tissue culture and rapid shell vial culture. To analyze whether CMV had a direct effect on the immunologic properties of kidney parenchymal cells, MHC class I and class II antigen expression was estimated before and after the infection. CMV infected all kidney cells identically. All cells expressed class I strongly after the infection, but they were class I positive prior to infection. Class II antigens were not expressed on the cell surface either before or after the infection. In conclusion, human kidney cells of glomerular, tubular and vascular origin were all infected by CMV without any difference. CMV had no significant direct effects on the antigenic properties of the cells.

Cytomegalovirus (CMV) infection is a common and often serious complication in renal transplant patients. A variety of clinical manifestations such as fever, leukopenia, retinitis, pneumonia and hepatitis have been described [1]. In addition, the virus may infect the kidney parenchyma and cause glomerulopathy [2]. A multicenter seroepidemiological study demonstrated that CMV infection impairs both patient and graft survival in renal transplantation [3].

Association between allograft rejection and CMV infection has been reported in several clinical series [4–6]. It has been suggested that CMV induces rejection by increasing the expression of MHC antigens in the graft [6, 7]. MHC class II antigens are especially important in the presentation of foreign antigens to alloreactive T lymphocytes, and their up regulation in vivo correlates with allograft rejection [8, 9]. The up regulation of class II antigens on the parenchymal cells of renal allografts has also been recorded during CMV infection [6]. The CMV-associated class II expression of kidney cells is thought to be mediated by γ-interferon, which is produced by activated T cells. However, it has been demonstrated in vitro that viral particles alone, without the presence of γ-interferon, are able to induce class II antigen expression on cell surfaces [10, 11]. It is also well known from DNA sequence analyses that CMV itself encodes molecules similar to MHC antigens [12] and that there is a sequence homology between HLA-DR beta chain and CMV immediate early antigens [13].

In this study, our aim was to analyze which structures of the kidney allograft would be the targets for CMV. Secondly, we wanted to study if the virus alone, without any additional immunological mechanisms, could change the expression of transplantation antigens in these structures. We investigated the ability of CMV to infect various types of cultured normal adult human kidney parenchymal cells in vitro. In addition, we analyzed the expression of HLA class I and class II antigens on the surface of cultured cells before and during CMV infection.

Methods

Isolation, culture and characterization of human kidney parenchymal cells

Isolation of different cell types. Normal human adult kidneys which could not be used for transplantation because of anomalies or other technical reasons, were obtained from the Division of Transplantation, Fourth Department of Surgery, University of Helsinki, Finland. Human glomerular epithelial and mesangial cells as well as tubular epithelial and endothelial cells were isolated by steel meshes of different pore size and enzymatic treatment using modifications [14] of the techniques of Burlington and Cronkite [15] and Kasten [16]. A whole kidney was first sliced with a scalpel and forced through a steel mesh, with 1000 mm pore size, with a syringe. The minced tissue was diluted with serum free MEM (Minimum essential medium, Gibco Ltd., Paisley, Scotland, UK), D-Valine was added instead of L-Valine to inhibit fibroblast growth [17], supplemented with 50 mg/ml gentamycin (Gibco) and 2 mmol/ml glutamine (Gibco). Tubular components were isolated by flushing the tissue through a steel mesh with 250 mm pore size. Glomerular components were collected after filtrating the supernatant through 100 mm and 75 mm pore size steel meshes. The isolated components were washed twice with serum free MEM. After centrifugation, one part of the tubular components was treated with 0.2 mg/ml of DNA:se (I, 600 IU/mg, Sigma Chemical Co., St. Louis, USA) and 0.2 mg/ml of collagenase (183 IU/mg, Worthington) in serum free MEM for 20 minutes at 37°C. The single cells were diluted in MEM with supplements and 10% fetal calf serum (FCS; Sera-Lab Limited, Sussex, England, UK) and plated in culture flasks at 37°C for two hours. The culture medium was then discarded and new medium was

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added for touched endothelial cells. After centrifugation, one part of the *glomerular components* was treated with type IV collagenase (Sigma; 750 IU/ml in 5 ml of serum free MEM, in 37°C and 5% CO_2 for 30 minutes, shaken every 5 min). After collagenase treatment the components were washed with MEM containing 20% FCS and centrifuged with 200 g for five minutes.

Cell cultures. Different cells were cultured in flasks (Nunclon, Copenhagen, Denmark) coated with collagen (Sigma; 10 mg/ml in PBS). Mesangial cells (enzyme treated glomerular components) were cultured in MEM supplemented with gentamycin, glutamine and 20% FCS. Tubular and glomerular epithelial cells (tubular and untreated glomerular components, respectively) as well as tubular endothelial cells were cultured in MEM supplemented with gentamycin, glutamine and 10% FCS. The cell cultures were usually confluent after two weeks. For detachment of the cells from the bottles, 5 ml versene (Gibco; 1:5000) was added. After a 15 minute incubation at 37°C the cells were washed through a 50 mm steel mesh to obtain single cell suspension, washed once with MEM and cultured again.

Characterization of different cell types. The origin of these cells was confirmed by light microscopy and morphological growth characteristics as well as by immunofluorescence (IF) stainings and specific antibodies. Glomerular and tubular epithelium exhibited typical epithelial morphology with phase contrast microscopy in cultures. Cells were polygonal, and formed a cobblestone-like monolayer when in confluence [18]. Mesangial cells were stellar, multilayered, and produced hillock formations [19]. Endothelial cells were identified by indirect IF staining only. To identify the various cell types in the cultures common specific markers were used: Factor VIII for endothelial cells, cytokeratin for epithelial cells and desmin for mesangial cells. Indirect IF staining for Factor VIII was performed with a rabbit anti-human Factor VIII antibody (Dakopatts, Copenhagen, Denmark), and only endothelial cell cultures were positive. A monoclonal anti-human desmin antibody (Labsystems, Helsinki, Finland) reacted only with mesangial cell cultures, and a monoclonal anti-human cytokeratin antibody (PKK-1, Labsystems) reacted only with epithelial cell cultures of glomerular and tubular origin. The specificity of the used monoclonal antibodies has been described [20]. According to positive reaction with specific antibodies, the purity of each cell population was over 90%.

Infection of cultured human kidney cells with cytomegalovirus

Human CMV strain AD169 (a laboratory strain) and a CMV strain isolated from human kidney transplant patient's urine were used in the study.

Conventional tissue culture. The conventional isolation of CMV was performed according to standard tissue culture techniques [21]. Appropriate CMV dilutions were inoculated onto human kidney cell monolayers. Cells were cultured in flasks containing tissue culture medium (MEM supplemented with glutamine, gentamycin and FCS). To detect the appearance of any cytopathic effect, the cultures were examined weekly after inoculation. In the case of a cytopathic effect the presence of CMV in the cell culture was demonstrated using a monoclonal antibody against CMV-specific early nuclear proteins (DuPont, Billerica, California, USA), and indirect immunofluorescence. To confirm the results, indirect immunoperoxidase staining and another monoclonal antibody against the immediate early antigens of CMV (Biotest Pharma, Frankfurt, Germany) were used. A modification [22] of the original immunoperoxidase method [23] to diagnose CMV was used.

Early-antigen immunofluorescence test. The rapid CMV shell vial culture and an early antigen immunofluorescence test were performed according to our own modification [24] of a method described previously [25]. In short, the isolated kidney cells were cultured to monolayers in MEM (supplemented with gentamycin, glutamine and FCS) in vials on a coverslip. CMV was inoculated onto cell monolayers by centrifugation with 3000 × g at 20°C for one hour, and the vials were incubated at 37°C with 5% CO_2. The culture medium was changed once a week. After 1 to 20 days of culture the cell monolayers were fixed with methanol at −20°C and stained with indirect immunofluorescence using a monoclonal IgG2a antibody against early CMV nuclear protein (DuPont) to demonstrate the presence of CMV. The results were confirmed by another monoclonal antibody against the immediate early antigens of CMV (Biotest Pharma) and indirect immunoperoxidase staining according to the method described [22, 23].

Demonstration of MHC antigens

Fluorescence-activated cell sorter (FACS) analysis. FACS analysis was performed to estimate the expression of class I and class II antigens on the cell surface of normal and CMV infected cells. The monoclonal antibodies against MHC class I (HLA-ABC, MAS 1523 c, Seralab, Sussex, England, UK) and MHC class II (HLA-DR, OKIa1, Ortho Pharmaceutical Co, Raritan, New Jersey, USA) were used. Cells were detached from culture flasks with versene and spun down. Single-cell suspensions were first exposed to a monoclonal antibody, washed twice and then exposed to a monoclonal fluorescein isothiocyanate conjugated anti-mouse antibody (GAM-FITC, Coulter Immunology, Hileah, Florida, USA), washed twice, resuspended in PBS and evaluated by the FACS.

Immunoperoxidase staining. For further analysis of MHC antigens in kidney cells, a three-layer immunoperoxidase technique [26] was performed for both shell vial cover slips and for cytocentrifuged cell preparations of the conventional cultures. The monoclonal antibodies against class I and class II antigens were the same as used in FACS analyses. The cell preparations were first incubated with a monoclonal mouse antibody, washed and incubated with peroxidase-conjugated rabbit anti-mouse antibody (Dakopatts). After washing, the cell smears were treated with peroxidase-conjugated goat anti-rabbit antibody (Tago, Burlingame, California, USA) and the reaction was revealed by AEC (3-amino-9-ethyl carbazole) solution containing hydrogen peroxide. Mayer's Hemalum was used for counterstaining.

Results

CMV infection

All cell material was isolated from five adult human kidneys and every experiment was repeated five times. Glomerular

mesangial and epithelial cell cultures as well as tubular epithelial cell and endothelial cell cultures were used in these experiments after four to five passages. Cells were positively infected with the CMV strain AD169 and with the strain from a renal transplant patient's urine but infection efficiency was dependent on the CMV strain (urine CMV was not as efficient as strain AD169, data not shown).

With the early-antigen immunofluorescence test on coverslips the infection with different CMV strains in mesangial cell and endothelial cell cultures was seen between days two and three, and in tubular epithelial cell and glomerular epithelial cell cultures between days three and four. With the conventional tissue culture method in culture flasks the first marks (+) of cytopathic effect (CPE) were seen after one to two weeks, but strong (+++) cytopathic effect was seen only after four weeks in all cell cultures. After CPE the positive infections were checked with antibodies towards immediate early antigens both with immunofluorescence and immunoperoxidase techniques (Fig. 1, Table 1).

HLA class I and class II expression

HLA class I and class II antigens on the cell surface were analyzed using a fluorescence activated cell sorter and monoclonal antibodies before and after CMV infection (Fig. 2). Before and after CMV infection in cultured human kidney endothelial cells, tubular epithelial as well as glomerular epithelial, and mesangial cells expressed class I antigen on their surface up to 94 to 99%. None of the tested cell types showed a positive binding with anti-class II antibody, neither before nor after infection with CMV. Thus, class II expression on the cell surface could not be demonstrated in CMV-infected kidney cell types.

While the FACS analyses demonstrated only the number of positive cells, the immunoperoxidase analyses showed the intensity of the staining, depending on the amount of the antigens in the cells. The results of the immunoperoxidase analyses are shown in Table 2. Before CMV infection, all cell types reacted with anti-class I antibody, but the intensity of the staining was slightly stronger after the infection. None of the cultured kidney cell types showed a positive staining with anti-class II antibody. When CMV infection was recorded in the cells, endothelial cells, mesangial cells and glomerular epithelial cells remained class II negative. Tubular epithelial cells from two kidneys showed a slight binding with the anti-class II antibody during CMV infection. However, this class II expression was obviously intracellular, as FACS analyses from the same cell populations showed no class II expression on the cell surface.

Discussion

This is the first study demonstrating that various cell types of a normal adult human kidney can be infected by CMV in vitro. As the cellular material was of normal kidney origin, our results were more comparable with the clinical situation than those of previous studies where embryonal tissues or laboratory cell lines were used as targets. The results support the suggestion that the renal allograft parenchyma may be infected with and even is a reservoir of CMV. Chronic viruria after transplantation can be explained by chronic infection of the organ. As all tested cell types of human kidney were infected, there was no evidence that certain structures in the organ would be particularly sensitive for CMV. Thus, the results demonstrate that the glomerular structures are not particular targets for the virus, although in the CMV associated glomerulopathy, other structures of kidney parenchyma remain intact [2].

CMV-associated glomerulopathy, which may occur in renal allografts without any evidence of rejection, may result from direct infection of glomerular structures. In addition to our investigation, previous studies on fetal tissues have shown that CMV can replicate in fetal mesangial cells [27], as well as in fetal glomerular epithelial cells [28] in vitro. However, also in the same study on fetal kidney, not only glomerular components but also tubular epithelium could be infected by CMV [28].

It has been shown that CMV can infect endothelial cell lines in vitro [7, 29–31]. The cells used in those experiments have usually been human umbilical venous endothelial cells (HUVE). In the present study, it was proven that CMV also infects endothelial cells of human kidney origin in vitro. The infected vascular structures have been earlier demonstrated in renal allograft biopsy and in autopsy materials only [32–34].

The relationship between MHC antigens and CMV is not yet totally understood, but it's importance is generally admitted. Induction of MHC class II antigen expression on renal allograft endothelial cells and tubular epithelial cells during CMV infection has been suggested to be a link between viral infection and rejection [6]. It has also been demonstrated that CMV increases the expression of class I antigens on the surface of endothelial cells [7, 31] and fibroblasts in vitro [35]. In addition, CMV can react with MHC class I antigens [36] and bind to beta$_2$-microglobulin [35]. Class I molecule is suggested to be a receptor for CMV [36].

In the present study, none of the cultured cell types expressed class II before the infection, not even on the endothelial cells which had been found class II positive in normal human kidney [37]. No class II antigens were demonstrated on the surfaces of CMV infected cells either. Although tubular epithelial cells showed a slight positive reaction with anti-class II antibody, the FACS analysis demonstrated no evidence of class II molecules on the cell surface. The positive reaction was obviously due to intracellular class II, also demonstrated in normal rat renal tubular epithelial cells [37]. The intensity of class I expression was slightly increased in the infected cells, but this finding is of minor significance, as all cells were class I positive prior to infection.

Class II expression is known to be upregulated by γ-interferon [38]. A mechanism has even been postulated in which γ-interferon initiates the reading of the coding sequences of the class II genome [39]. It was postulated that in the cells normally not expressing class II but also considered as "interferon-γ-inducible cells," both the promoter and the transcriptional enhancer are inactive, and no gene transcription is seen. However, after administration of γ-interferon, the promoter is activated, and the HLA-DRa gene is transcribed: the repressors acting at the promoter can be modified through the action of γ-interferon-inducible factors [39]. Interferon-γ is produced by activated lymphocytes not only during rejection but also during viral infections [38, 40], and it has been suggested that the CMV-induced class II expression is mediated by γ-interferon, which is known to up regulate class II antigen. However, viral

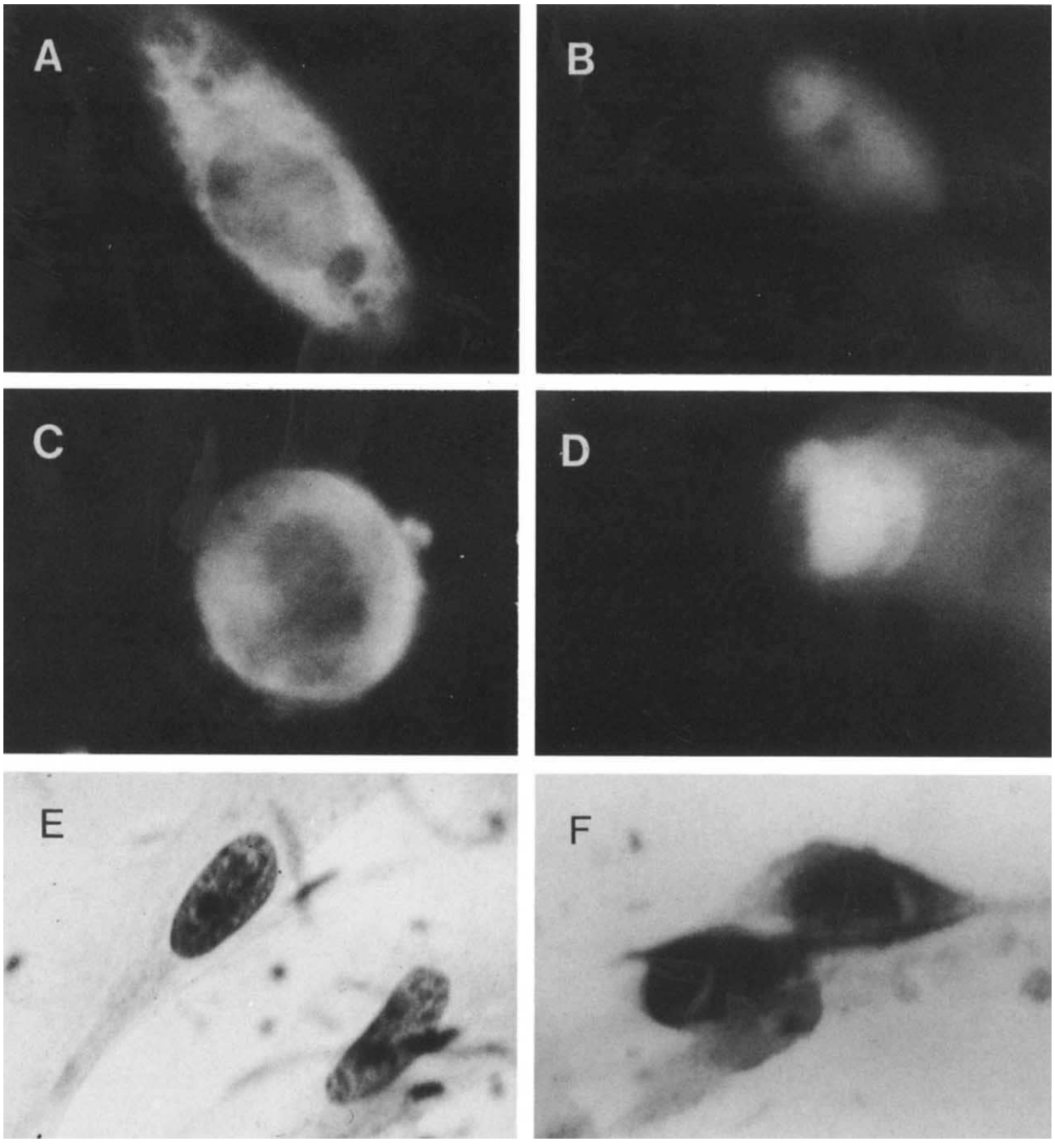

Fig. 1. *Various kidney cell types.* **A.** An endothelial cell in the culture monolayer identified by anti-factor VIII antibody and indirect immunofluorescence (note the positive reaction in the cytoplasm). **B.** A CMV-positive endothelial cell demonstrated by a monoclonal antibody against the early nuclear proteins of CMV (note the positive reaction in the nucleus). **C.** A glomerular epithelial cell from a conventional culture identified by anti-cytokeratin antibody. **D.** A CMV-infected glomerular epithelial cell demonstrated as above. **E.** Tubular epithelial cells in tissue culture before CMV infection. **F.** Infected tubular cells demonstrated by a monoclonal antibody against immediate early antigens of CMV and immunoperoxidase staining (note the positive staining of nuclei and inclusion bodies).

particles alone, in the absence of γ-interferon, are able to induce class II antigen expression on some cells [10, 11]. Also recent DNA sequence analyses have demonstrated that CMV encodes a molecule similar to MHC class I [12], and that there is sequence homology and immunological cross reactivity between HLA-DRβ chain and CMV immediate early antigens [13].

As CMV did not induce class II on kidney cells in vitro, it is more likely that class II expression associated with CMV in vivo is caused by lymphocyte activation and γ-interferon than

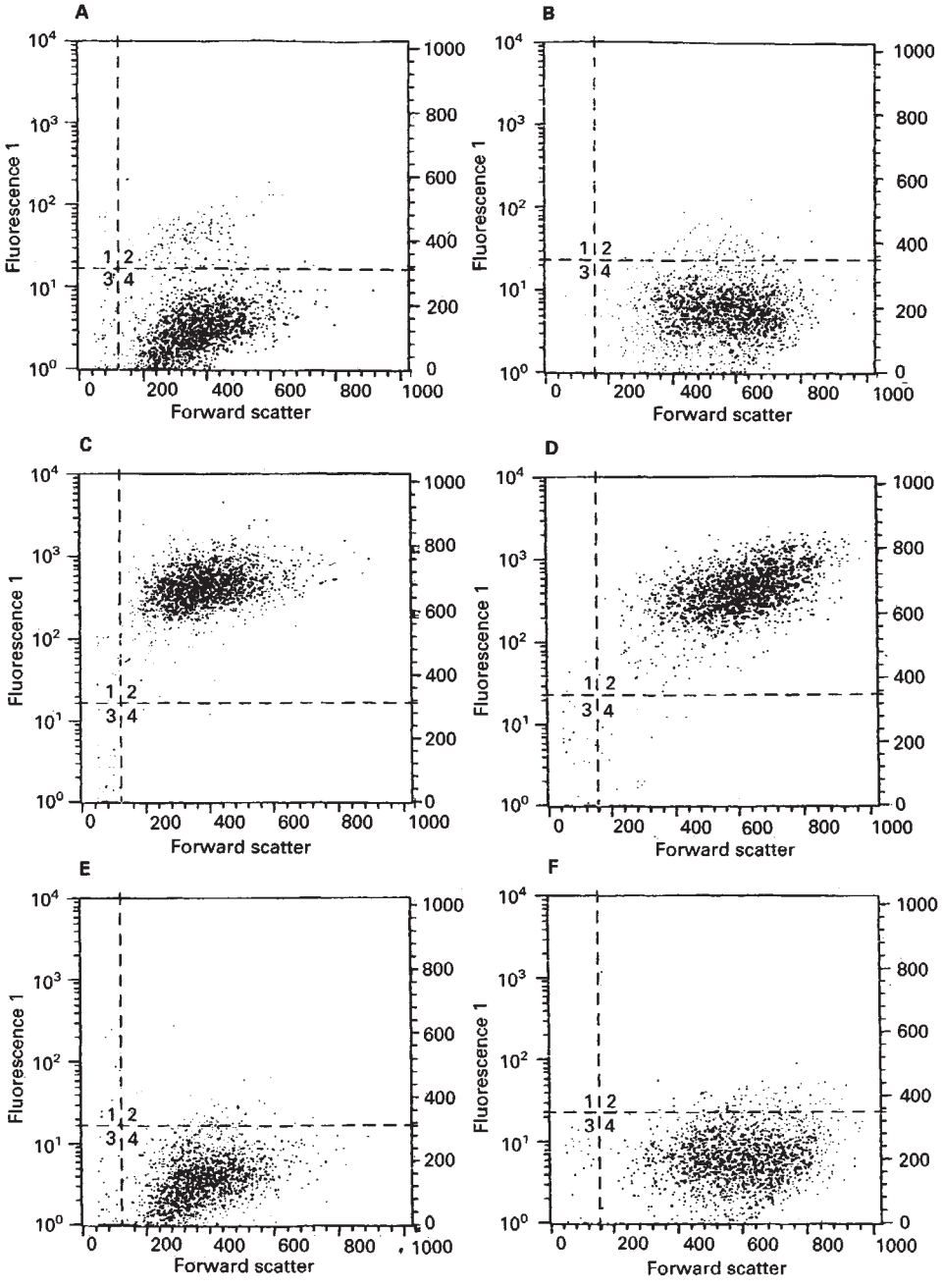

Fig. 2. *Fluorescence activated cell sorter (FACS) analysis of MHC class I and class II antigens on the cell surface of normal and CMV infected mesangial cells.* **A.** A negative control (background binding) before (7%) and **(B)** during CMV infection (after 30 days in culture) (4%). **C.** Class I positive cells before (96%) and **(D)** during the infection (98%). **E.** The reactivity of the cells with anti-class II antibody exceeded the background binding neither before (3%) nor **(F)** during infection (3%).

Table 1. Infection of human kidney cell cultures with CMV strain AD169

Cell type	ea-IF days	CPE (+++) days
MC	2–3	28
GEPC	3–4	28
EC	2–3	28
TEPC	3–4	30

All results are given in days. Early-antigen immunofluorescence test (ea-IF) was done on coverslips and cytopathic effect (CPE) was detected in culture flasks. Abbreviations are: MC, mesangial cell; GEPC, glomerular epithelial cell; EC, endothelial cell; TEPC, tubular epithelial cell.

Table 2. HLA class I and class II antigen expression before and during CMV infection as demonstrated by immunoperoxidase staining

Cell type	Before infection		During infection	
	Class I	Class II	Class I	Class II
MC	++	–	+++	–
GEPC	++	–	+++	–
EC	++	–	+++	–
TEPC	++	–	+++	(+)

Intensity of the staining is estimated with a scale from – to +++, where +++ is the most intensive staining. Abbreviations are in Table 1.

by the virus itself. On the other hand, the immunological cross reactivity may be also involved in the mechanisms in vivo, while the molecules coded by CMV are similar to the MHC antigens. It is possible that the immunological response against the MHC antigens in the kidney allograft is cross reacting with the molecules coded by CMV and produced in the infected cells. To understand the association between renal allograft rejection and CMV infection thoroughly, the specificity of the immune response should also be studied.

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References

1. Rubin RH: Infection in the renal and liver transplant patient, in *Clinical Approach to Infections in the Compromised Host* (2nd ed), edited by Rubin RH, Young LS, New York, Plenum Press, 1988, p. 557
2. Richardson WP, Colvin RB, Cheeseman SH, Tolkoff-Rubin NE, Herrin JT, Cosimi AB, Collins AB, Hirsch MS, McCluskey RT, Russell PS, Rubin RH: Glomerulopathy associated with cytomegalovirus viremia in renal allografts. *N Engl J Med* 305:57–63, 1981
3. Rubin RH, Tolkoff-Rubin NE, Oliver D, Rota TR, Hamilton J, Betts RF, Pass RF, Hillis W, Szmuness W, Farrell ML, Hirsch MS: Multicenter seroepidemiologic study of the impact of cytomegalovirus infection on renal transplantation. *Transplantation* 40:243–249, 1985
4. Simmons RL, Weil R, Tallent MB, Kjellstrand CM, Najarian JS: Do mild infections trigger the rejection of renal allografts? *Transplant Proc* II:419–423, 1970
5. Lopez C, Simmons RL, Mauer SM, Najarian JS, Good RA: Association of renal allograft rejection with virus infection. *Am J Med* 56:280–289, 1974
6. Von Willebrand E, Pettersson E, Ahonen J, Häyry P: CMV infection, class II antigen expression, and human kidney allograft rejection. *Transplantation* 42:364–367, 1986
7. Van Dorp WT, Jonges E, Bruggeman CA, Daha MR, van Es La, van der Woude FJ: Direct induction of MHC class I, but not class II, expression on endothelial cells by cytomegalovirus infection. *Transplantation* 48:469–472, 1989
8. Hall BM, Bishop GA, Duggin GG, Horvath JS, Philips J, Tiller DJ: Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: Relevance to the rejection response. *Lancet* II:247–251, 1984
9. Henny FC, Weening JJ, Baldwin WM, Oljans PJ, Tanke HJ, van Es LA, Paul LC: Expression of HLA-DR antigens on peripheral blood T lymphocytes and renal graft tubular epithelial cells in association with rejection. *Transplantation* 42:479–483, 1986
10. Massa PT, Dörries R, Ter Meulen V: Viral particles induce Ia antigen expression on astrocytes. *Nature* 320:543–546, 1986
11. Kannagi M, Kiyotaki M, King NW, Lord CI, Letvin NL: Simian immunodeficiency virus induces expression of class II major histocombatibility complex structures on infected target cells in vitro. *J Virol* 61:1421–1426, 1987
12. Beck S, Barrell BG: Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. *Nature* 331:269–272, 1988
13. Fujinami RS, Nelson JA, Walker L, Oldstone MB: Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DR b chain: A means for graft rejection and immunosuppression. *J Virol* 62:100–105, 1988
14. Mattila PM, Nietosvaara YA, Ustinov JA, Renkonen RL, Häyry PJ: Antigen expression in different parenchymal cell types of rat kidney and heart. *Kidney Int* 36:228–233, 1989
15. Burlington H, Cronkite EP: Characteristics of cell cultures derived from renal glomeruli. *Proc Soc Exp Biol Med* 142:143–149, 1973
16. Kasten FH: Rat myocardial cells in vitro: Mitosis and differentiated properties. *In Vitro* 8:128–149, 1972
17. Gilbert SF, Migeon BR: D-Valine as a selective agent for normal human and rodent epithelial cell culture. *Cell* 5:11–17, 1975
18. Detrisac CJ, Sens MA, Garvin AJ, Spicer SS, Sens DA: Tissue culture of human kidney epithelial cells of proximal tubule origin. *Kidney Int* 25:383–390, 1984
19. Striker GG, Keller PD, Faren FM: Human glomerular cells in vitro: Isolation and characterization. *Transplant Proc* 7:88–89, 1980
20. Virtanen I, Lehto VP, Lehtonen E, Vartio T, Stenman S, Kurki P, Wager O, Small JV, Dahl D, Badley RA: Expression of intermediate filaments in cultured cells. *J Cell Sci* 50:45–63, 1981
21. Arvin AM: Cytomegaloviruses, in: *Laboratory diagnosis of Viral Infections*, edited by Lennette EH, New York, Marcel Dekker, 1985, p. 225
22. Lautenschlager I, Ahonen J, Eklund B, Höckerstedt K, Isoniemi H, Salmela K, Häyry P: Detection of cytomegalovirus-specific early antigens from blood leukocytes and bronchoalveolar lavage cells in the diagnosis of active cytomegalovirus infection in transplant patients. (abstract) *Transplant Proc* 22:233, 1990
23. van der Bij W, Torensma R, van Son WJ, Anema J, Schirm J, Tegzess AM, The TH: Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *J Med Virol* 25:179–188, 1988
24. Lautenschlager I, Suni J, Ahonen J, Grönhagen-Riska C, Ruutu P, Ruutu T, Tukiainen P: Detection of cytomegalovirus by the early-antigen immunofluorescence test versus conventional tissue culture. *Eur J Clin Microbiol Infect Dis* 8:610–613, 1989
25. Smith TF: Rapid methods for diagnosis of viral infections. *Lab Med* 18:16–20, 1987
26. von Willebrand E, Zola H, Häyry P: Thrombocyte aggregates in renal allografts. Analysis by the fine needle aspiration biopsy and monoclonal anti-thrombocyte antibodies. *Transplantation* 39:258–262, 1985
27. Heieren MH, van der Woude FJ, Balfour HH: Cytomegalovirus replicates efficiently in human kidney mesangial cells. *Proc Natl Acad Sci USA* 85:1642–1646, 1988
28. Heieren MH, Kim Y, Balfour HH: Human cytomegalovirus infection of kidney glomerular visceral epithelial and tubular epithelial cells in culture. *Transplantation* 46:426–432, 1988
29. Ho DD, Rota TR, Andrews CA, Hirsch MS: Replication of human cytomegalovirus in endothelial cells. *J Infect Dis* 150:956–957, 1984
30. Smiley ML, Mar E-C, Huang E-S: Cytomegalovirus infection and viral-induced transformation of human endothelial cells. *J Med Virol* 25:213–226, 1988
31. Sedmak DD, Roberts WH, Stephens RE, Buesching WJ, Morgan LA, Davis DH, Waldman WJ: Inability of cytomegalovirus infection of cultured endothelial cells to induce HLA class II antigen expression. *Transplantation* 49:458–462, 1990
32. Payton D, Thorner P, Eddy A, Yeger H, Baumel R: Demonstration by light microscopy of cytomegalovirus on a renal biopsy of a renal allograft recipient. *Nephron* 47:205–208, 1987
33. Macasaet FF, Holley KE, Smith TF, Keys TF: Cytomegalovirus studies of autopsy tissue. II Incidence of inclusion bodies and related pathologic data. *AJCP* 63:859–865, 1975
34. Myerson D, Hackman RC, Nelson JA, Ward DC, McDougall JK: Widespread presence of histologically occult cytomegalovirus. *Hum Pathol* 15:430–439, 1984
35. Grundy JE, Ayles HM, McKeating JA, Butcher RG, Griffiths PD, Poulter LW: Enhancement of class I HLA antigen expression by cytomegalovirus: Role in amplification of virus infection. *J Med Virol* 25:483–495, 1988
36. Grundy JE, McKeating JA, Ward PJ, Sanderson AR, Griffiths PD: β_2 microglobulin exchanges the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. *J Gen Virol* 68:793–803, 1987

37. VON WILLEBRAND E, LAUTENSCHLAGER I, INKINEN K, LEHTO V-P, VIRTANEN I, HÄYRY P: Distribution of the major histocompatibility complex antigens in human and rat kidney. *Kidney Int* 27:616–621, 1985
38. POBER JS, GIMBRONE MA, CONTRAN RS, REISS CS, BURAKOFF SJ, FIERS W, AULT KA: Ia expression by vascular endothelium is inducible by activated T cells and by human γ interferon. *J Exp Med* 157:1339–1353, 1983
39. SULLIVAN KE, CALMAN AF, NAKANISHI M, TSANG SY, WAND Y, PETERLIN BM: A model for the transcriptional regulation of MHC class II genes. *Immunol Today* 8:289–293, 1987
40. MAUDSLEY DJ, MORRIS AG, TOMKINS P: Regulation by interferon of the immune response to viruses via the major histocompatibility complex antigens, in *Immune Responses, Virus Infections and Disease*, edited by DIMMOCK NJ, MINOR PD, Oxford, IRL Press at Oxford University Press, 1989, p. 15