Priority Paper Evaluation
Future Microbiology

Endothelial cells and CMV dissemination

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Evaluation of: Sacher T, Andrassy J, Kalnins A *et al.* Shedding light on the elusive role of endothelial cells in cytomegalovirus dissemination. *PLoS Pathog.* 7(11), E1002366 (2011). Using the murine CMV animal model and the well-established model of *Cre*-lox-P-mediated green-fluorescence tagging of endothelial cell (EC)-derived mouse CMV to quantify the role of infected ECs in transplantation-associated CMV dissemination (in mice expressing *Cre* recombinase under the control of either the Tie2 or the Tek promoter selectively expressed in vascular EC-Tie-*Cre* and Tek-*Cre* mice), it was shown that EC-derived virus contributed to 50% of the total viral load during primary infection, and there was no preference for dissemination of EC-derived viruses over viruses produced by other cell types. In addition, during secondary viremia, there was only a negligible contribution of EC-derived virus to dissemination to other organs. These results are novel in the methodology employed and are somewhat interesting. However, the data are limited to the mouse model with a short-term follow-up, and the immunodeficient host has not yet been studied. In humans, these conclusions must be taken with caution. First, in primary infection occurring through natural routes, epithelial cells are infected first, then ECs, unless primary infection occurs through blood transfusion, in which case endothelial vascular cells may become infected first. In both cases, the virus transport occurs through the intervention of leukocytes, namely monocytes and polymorphonuclear leukocytes. As monocytes differentiate to macrophages, they become highly susceptible to human CMV replication inside organ tissues, while polymorphonuclear leukocytes are active in virus capturing from infected endothelial vascular cells and transporting to distant sites.

Summary of methods & results

In this manuscript, Sacher *et al.* [1] challenge the model developed more than 60 years ago by Frank Fenner [2]. This model proposed a two-step dissemination pattern for systemic virus infections. Primary viremia would transport the virus from the site of entry to internal organs, such as the liver and spleen, where the virus replicates, and secondary viremia would cause dissemination from internal organs throughout the body. This model was accepted for many years and for many viruses, until recently, when it was shown that viruses produced in hepatocytes are locally disseminated to other cell types, but are not distributed from the liver to other organs via secondary viremia [3]. By representing the boundary between blood and organ parenchyma, endothelial cells (ECs) are currently considered susceptible to bidirectional virus spread to and from a transplanted organ.

Sacher *et al.* developed a method od *Cre*-lox-P-mediated green-fluorescence tagging of EC-derived murine CMV (MCMV) [1] to label viruses in defined cell types *in vivo* and then trace the viral progeny of that cell type [3]. To study the role of ECs in MCMV replication, an MCMV mutant was used that contains a *Cre*-inducible *egfp* expression cassette (MCMV-flox). Mice expressing *Cre* recombinase under the control of either the Tie2 or Tek promoter (which are selectively expressed in vascular ECs) were infected with MCMV-flox. During virus replication in ECs only, MCMV-flox is efficiently recombined to MCMV-*Cre*. The resulting recombinant virus is stable.

Using this methodology, EC-derived viruses contributed to 50% of the total virus load during primary infection. However, there was no preference for dissemination of EC-derived viruses over those produced by other cell types. Furthermore, in secondary infections, EC-derived viruses were poorly disseminated via secondary viremia. In other words, primary viremia entails an important (although not exclusive) contribution of EC-derived viruses for dissemination, whereas secondary viremia plays only a marginal role in EC-derived virus dissemination into a transplanted uninfected organ or from an infected to other uninfected organs. Only cell-free virus preparations inoculated intravenously were able to infect multiple organs at a high titer.

Keywords

Future Medicine part of fsg

Discussion

The results reported in the manuscript by Sacher *et al.* on the elusive role of ECs in CMV dissemination may have some interest because they shed light on the role of EC-derived MCMV in virus dissemination in the mouse model [1]. The original methodology used allows us to trace EC-derived viruses in different organs. However, as the authors point out, the possibility of tracing the virus is rather short-lived and does not allow for the study of late phases of experimental infection. In addition, transplantation experiments were only performed in immunocompetent, and not immunosuppressed mice. Thus, these two conditions substantially limit the conclusions of the study.

It appears pertinent to discuss the results obtained in the experimental mouse model within the context of current knowledge on the dissemination of human CMV (HCMV) in immunosuppressed transplant patients. The AIDS experience in the 1980s has shown that HIV-infected patients with AIDS often died with a disseminated HCMV infection associated with a high HCMV load in blood [4]. HCMV presence in blood was initially shown by virus isolation from peripheral blood leukocytes according to the conventional procedure using human embryonic lung fibroblasts or the rapid method based on the shell–vial technique.

However, at the end of the 1980s, a new procedure was developed simultaneously in Groningen, The Netherlands [5] and in Pavia, Italy [6]. This new procedure was referred to as the HCMV antigenemia assay and was based on the detection of leukocytes (polymorphonuclear leukocytes and monocyte/macrophages) carrying HCMV pp65 in the nucleus by an immunohistochemical technique (either the immunofluorescence or immunoperoxidase technique) [7,8]. In the following years, after several unsuccessful attempts, we were able to reproduce *in vitro* the pp65 antigenemia assay by coculturing polymorphonuclear leukocytes and monocytes/macrophages from healthy blood donors with human umbilical vein ECs (HUVECs) infected with recently isolated HCMV strains [9]. In other words, peripheral blood leukocytes could be infected and these carried HCMV pp65 only when cocultured with HUVECs infected with wild-type HCMV strains and not with laboratory-adapted HCMV strains, such as AD169 and Towne [10]. In addition, we could show that the pathogenetic basis of disseminated HCMV infection (i.e., antigenemia) was due to the transfer of viruses and viral products from infected HUVECs to leukocytes [11]. This process was mediated by a five-protein (pentamer) complex (gH/gL/pUL131-128) present on the envelope of virus particles and required for infection of both ECs and epithelial cells [12–14].

The same five-protein complex was involved in virus transfer from HUVECs to leukocytes and virus cell-to-cell spreading (plaque formation), as shown by inhibition of both events with a number of pentamer-reactive human monoclonal antibodies possessing a potent neutralizing activity [15] [Manuscript in Preparation].

When we tried to isolate cell-free viruses from the plasma of patients with AIDS and disseminated HCMV infection, we consistently failed. It is plausible that cell-free viruses released into the bloodstream by infected ECs was soon neutralized by circulating antibodies. Thus, viral load in the maximally immunocompromised patients (AIDS patients) consisted of leukocytes infected through adhesion with infected ECs [11,16]. In addition, viral load was also integrated by infected ECs detaching from the vessel walls and being released into the bloodstream [17,18].

In conclusion, infected ECs appear to be the major source of viral load in blood through the transfer of viruses and viral products to circulating leukocytes. In humans, this event appears mostly to occur in reactivated HCMV infections, where ECs act as a HCMV reservoir. Viruses are transported by leukocytes, which can infect ECs, epithelial cells and fibroblast cells of different organs at distant sites [19]. This process, given the limited possibility of tracing EC-derived viruses in the mouse model, may only be apparently different from that reported for the mouse model.

Future perspective

In the next 5-years, the role of ECs in the pathogenesis of HCMV infection in humans will be better defined. Data acquired thus far suggest that in the pathogenesis of both disseminated infection of the immunocompromised patients and congenital HCMV infection as well as primary and reactivated infection of immunocompetent subjects, ECs are primarily involved. In addition, the control of HCMV dissemination by the immune response will be elucidated at the level of both the humoral and T-cell-mediated arm. In particular, the interplay of antibodies and the $CD4^+$ and $CD8^+$ T-cell response will be better clarified with respect to present knowledge, in which the interaction of the two arms of the immune response remains to be investigated in more depth.

Executive summary

- It has been shown in the mouse model that endothelial cells (ECs) contribute to murine CMV (MCMV) dissemination by approximately 50% in cases of primary infection, whereas their contribution to MCMV dissemination in secondary infection is negligible.
- The methods used in the evaluated study to trace EC-derived MCMV appear original and reliable; however, the allow only short-term tracing of EC-derived viruses.
- In addition, experiments were performed in immunocompetent, and not immunocompromised animals, where the dynamics of EC contribution to virus dissemination may differ greatly.
- It is concluded that in the mouse model with secondary viremia, MCMV dissemination is cell-associated; however, which types of cells are involved was not determined.
- In humans, namely in immunocompromised patients and in newborns with congenital human CMV (HCMV) infection, vascular ECs are a major virus reservoir and, following adhesion of circulating leukocytes, they transfer HCMV and HCMV products to both polymorphonuclear leukocytes and monocytes/macrophages. Then, leukocytes detach from infected ECs entering the bloodstream and may adhere to uninfected ECs, transferring to them HCMV and HCMV products, or may enter organ tissues, disseminating the infection locally to epithelial cells and fibroblasts.
- Thus, in humans, the role of ECs in HCMV dissemination appears dominant, in that ECs continuously replicate the virus, which is transferred to leukocytes.
- In addition, some infected ECs, upon becoming cytomegalic, may detach from the vascular wall, enter the bloodstream and transport the virus to distant sites.
- In conclusion, in humans, dissemination of HCMV infection seems to be supported by circulating leukocytes carrying infectious virus and viral products transferred by infected vascular ECs. In immunocompetent subjects, HCMV may disseminate more rapidly due to the initial lack of the immune response in primary infection, whereas in reactivated infections, the immune control prevents dissemination. In the immunocompromised host, immune control during reactivated infections may be partially or totally deficient, thus facilitating more extensive virus dissemination.

Financial & competing interests disclosure

This work was partially supported by Fondazione C Denegri, Torino, Italy. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Writing assistance was utilized in the production of this manuscript. The author is also indebted to D Sartori for editing the manuscript and to L Kelly for revising the English.

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- ▪ of interest
- ▪▪ of considerable interest

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