Polymorphonuclear Leukocytes Mainly Contribute to Human Herpesvirus–6 Load in the Peripheral Blood of Patients

To the Editor—Human herpesvirus–6 (HHV-6) is a β-herpesvirus closely related to human cytomegalovirus. Because of the preferential lymphotropism of HHV-6 in vivo [1], the diagnosis of HHV-6 infection is based on the detection and quantitation of a viral genome in PBMCs, while the diagnosis of human cytomegalovirus mainly relies on the study of peripheral blood polymorphonuclear leukocytes (PMNLs). However, the mechanisms explaining the presence of human cytomegalovirus in PMNLs, either full replication or simple passive transport, remain a matter for discussion [2]. Except for the findings of the study by Luppi et al. [3], which reported the detection of an integrated HHV-6 genome both in peripheral blood PMNLs and in bone marrow granulocyte precursors in 3 patients, little is known about the role of PMNLs in HHV-6 infection. Their ability to support HHV-6 replication and their potential role in viral cell-to-cell transmission, as well as their value as markers of infection, are still pending questions.

As a first approach, we evaluated HHV-6 DNA load in paired PMNL and PBMC samples obtained from 20 patients with suspected HHV-6 infection. PMNLs and PBMCs were isolated with the anti-CD15 magnetic bead purification method and Ficoll gradient separation, respectively. The HHV-6 genome was detected more frequently in PMNLs (in 18 of 20 samples) than in PBMCs (in 9 of 20 samples). Moreover, the median HHV-6 DNA load was higher in PMNLs than in PBMCs (2049 copies/10^6 cells vs. 120 copies/10^6 cells (figure 1A).

To investigate whether PMNLs could constitute target cells for HHV-6 replication and transmission, we developed a PMNL-PBMC coculture system in vitro. Samples of both leukocyte subpopulations were obtained from healthy donors using dextran or Ficoll gradient separation on peripheral blood for PMNLs and PBMCs, respectively. Prior to cell coculture, PBMCs were infected with either U1102 (variant A) or MAR (variant B) HHV-6 strains. By day 7 after infection, when the immunofluorescent staining with a specific anti–HHV-6 monoclonal antibody was >80%, PMNLs were added to PBMC culture (ratio, 1:1). Sixty hours later, the 2 leukocyte subpopulations were again purified, and HHV-6 DNA load was measured in each of them. Similar to what was observed in vivo, HHV-6 load was higher in PMNLs than in PBMCs for both variants, as illustrated in figure 1B.

Figure 1. Human herpesvirus–6 (HHV-6) DNA load (logarithmic scales) in peripheral blood polymorphonuclear leukocytes (PMNLs) and PBMCs. A, real-time PCR was performed with paired PMNL and PBMC samples obtained from peripheral blood samples of 20 patients. Results are represented as median values. B, Real-time PCR was performed with PMNL and PBMC samples from in vitro cocultures, as described. For each viral strain, coculture experiments were performed in triplicate. Results are represented as mean values, and the error bars indicate SDs.
According to these results, PMNLs seem to harbor the majority of HHV-6 DNA load in vivo (in the peripheral blood of patients) and in vitro (in the PMNL-PBMC coculture system described here). Consequently, to improve the sensitivity of HHV-6 diagnosis among patients, a logical proposal is to measure HHV-6 DNA load in whole blood samples rather than in purified PBMCs, as previously reported for human cytomegalovirus [4]. In addition, the PMNL-PBMC coculture system will permit further investigation of whether the high HHV-6 load in PMNLs reflects the ability of HHV-6 to replicate in these cells or is the consequence of a phagocytic process.

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References


Methicillin Resistance and Its Relation to Prognosis in Patients with Ventilator-Associated Pneumonia: Still an Unsolved Issue

To the Editor—Zahar et al. [1] addressed some unsolved issues in the evaluation of prognosis of patients with ventilator-associated pneumonia (VAP) due to methicillin-resistance Staphylococcus aureus (MRSA) infection. The controversial findings of previous studies have been ascribed to limitations in sample selection and in size, as well as to confounding [2,3]. Zahar et al. [1] tried to overcome some of these limitations and presented the largest study comparing the prognosis of patients with VAP due to methicillin-resistant S. aureus (MSSA). By controlling for various potential confounding factors, they showed that infection due to MRSA was not associated with higher risk of death, adding one more piece to our understanding of this complex relationship. We believe, however, that their study [1] still contains some of the limitations typical of observational studies. Their selection of confounding variables seems to be quite arbitrary. Stratification for length of stay in an intensive care unit is adequate, because it influences markedly the risk of death. Other variables, however, were chosen in view of their unbalanced distribution at the time of admission to the intensive care unit. Procedures to select confounding variables have been debated, and a priori definition of confounding variables on the basis of some theoretical model has been recommended. Hierarchical approaches are another option [5]. Despite being the largest study to address this topic, the study by Zahar et al. [1] is not large enough to ensure that the difference does not exist (i.e., to reject the null hypothesis), and it thereby incurs a type II error [4]. The power of the study to reject the null hypothesis for the hospital mortality outcome was only 62%.

The importance of infection due to MRSA in patients with VAP is still an unsolved issue. Prospectively planned studies (with standardized protocols to be used in all intensive care units that are involved in data collection) are required. In the meantime, measures to control the widespread prevalence of infection due to MRSA must be undertaken.

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