

Reply to Sauvage et al

TO THE EDITOR—We thank Sauvage et al for their correspondence. We would first like to emphasize that the possibility of false-negative polymerase chain reaction (PCR) results is another hypothesis that the authors have not explored. Indeed, several reasons, such as poor conservation conditions or long conservation periods for the plasma or extracted nucleic acids and/or low copy numbers of the viral genome in the original sample, might also explain this negative result. In our experience with the detection of Marseillevirus-like virus DNA in serum or plasma samples from blood donors, PCR signals are very weak in the original samples (ie, samples in which viral particles from large volumes have not been concentrated) and close to the lower limit of detection, suggesting that a possible latent infection with episodic reactivation, as is the case for several herpesviruses, needs to be further investigated.

In contrast, the PCR signal was very strong in the most evident case of primary infection with a Marseillevirus-like virus that we have observed to date; this case occurred in an 11-month-old boy with adenitis and was reported elsewhere [1]. Unfortunately, these results were not detailed in the correspondence from Sauvage et al. Indeed, in the case report from *The Journal of Infectious Diseases*, published later in 2013 [2], Marseillevirus-like virus infection was detected by immunoassays, PCR/sequencing analysis, immunofluorescence (IF), fluorescence in situ hybridization (FISH), immuno-

chemistry, and 2-dimensional Western blotting. In particular, at the time of adenitis, the patient's serum was strongly positive by PCR. The sequence recovered by PCR was unique, differing from that of other Marseillevirus strains. One year later, the serum was negative for Marseillevirus-like virus DNA by PCR. In addition, enzyme-linked immunosorbent assay detected high anti-Marseillevirus immunoglobulin G (IgG) and immunoglobulin M (IgM) titers in a blood serum specimen from the patient. One year later, the IgG titer remains high, whereas the IgM response has decreased dramatically. The serum also exhibited a strong reactive signal against Marseillevirus proteins (the strongest of which was observed against the capsid protein) on a 2-dimensional Western blot.

Last but not least, using immunohistochemistry and combined IF/FISH (with a DNA probe targeting a different region than that amplified by PCR when analyzing the serum), we identified Marseillevirus antigen and DNA in sections of a lymph node specimen obtained from the patient.

It is very difficult to believe that laboratory contamination can explain the strong and specific intracellular signal obtained in the lymph node sections, given that neither the lymph node paraffinization nor the sample sectioning was performed in the laboratory, but rather at the hospital. Similarly, all immunohistochemistry experiments were performed by the histological service of Timone Hospital in Marseille, France.

On the basis of our experience with PCR results, one should be prudent with regard to both positive and negative results. Indeed, PCR-positive results must be confirmed using other primer sets (as was the case for primers ORF152 and ORF268 in the original blood donor study [2]), using other techniques (such as serology, microscopy, FISH, IF, immunohistochemistry, whole-genome sequencing, and proteomics, as in our previous studies [1–3]), and by other laboratories. Of note, independent of our work, Gilbert

Greub's team in Lausanne detected Lausannevirus, another Marseillevirus, with a seroprevalence ranging from 1.74% to 2.51% in healthy Swiss men [4].

Additionally, PCR-negative results should be considered with great care before decreeing that all previously performed work resulted from laboratory contamination.

Because we have found several other individuals infected by a Marseillevirus-like virus, we would be pleased to send the authors positive samples (plasma or extracted nucleic acids). Further collaborative work will help virologists and clinicians to better understand the origin, fate, and consequences of Marseillevirus-like virus infection in humans.

Notes

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