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CORRESPONDENCE

Letter to Editor

To the Editor: In the article titled "Myocardial inflammation, cellular death, and viral detection in sudden infant death caused by SIDS, suffocation, or myocarditis," Krous *et al.* (1) presented results concerning cases of suspected SIDS ($n = 24$), suffocation ($n = 25$), and myocarditis ($n = 4$). Their data indeed did not confirm the findings of our study published in this journal (2), possibly due to technical and analytical differences between the two studies. We reported an increase in the number of T lymphocytes in cases of suspected sudden infant death syndrome (SIDS) ($n = 63$) compared with controls who had died of non-natural causes ($n = 11$). In addition, we found viral genome in myocardial samples of SIDS cases (fixed up to only 48 h in neutral phosphate-buffered formaldehyde). In our prospective study, we used another antibody than that used by Krous *et al.* to detect T lymphocytes (CD45R0 instead of CD3) to identify cases suspicious for inflammation. On the one hand, our immunohistochemical preliminary criteria (2) are more rigorous compared with those suggested for adults and on the other hand, the interstitial space in the myocardium of babies is less than in the myocardium of adults. Therefore, our suggestions are not arbitrary and >10 $CD45R0^+$ T lymphocytes or >15 $CD45R0^+$ T lymphocytes and $CD68^+$ macrophages in summation (mean value of 20 high-power fields; $\times 400$), found in the myocardial interstitium, demonstrate an inflammatory myocardial process. The presence of either myocardial inflammatory infiltrates or single focal inflammatory infiltration only demonstrates the necessity to investigate serial samples. Are there really only a few scattered inflammatory cells and necrotic cardiomyocytes, regardable as not pathologic and found by chance?

If a mild or moderate increase of T lymphocytes and evidence of, for example, an enteroviral genome (well known to be able to cause lethal arrhythmias and to infect cardiomyocytes), the T lymphocytes seem to indicate an inflammatory process, even if the Dallas criteria for myocarditis are not met. Indeed, pathologists have yet to agree on what constitutes myocarditis and Krous *et al.* mention a number of criteria. One criterion must be the detection of viral genome by PCR techniques because virus infections are the most common cause of myocarditis. Meanwhile, we found the same results in a greater study group and control group (3). Other working groups also attributed cases of sudden children death to virus infections in the context of SIDS (4,5). With regard to the fact that infections with the viruses detected (enteroviruses, adenoviruses, Epstein-Barr-virus, cytomegalovirus, human herpes simplex virus type 6, and parvovirus B19) are common in early childhood, the detection of one of these viruses also in one control case is possible, the myocardium is a key target tissue for especially coxsackievirus infection, but it is not the only virus, the viruses seem to trigger sudden cardiac death: our hypothesis is, that unknown constitutional or genetic risk factors (*e.g.*, cardiomyopathies, misdiagnosed as SIDS, differences in the immune response of the organism to viral antigens) together with environmental circumstances (sleeping position, room temperature, *etc*.) can lead to sudden cardiac death, which may occur due to virus infection in combination with *e.g.*, genetic heart disorders associated with structural and arrhythmogenic abnormalities. It should be noted that, as far as we know, the human myocardium cannot be regarded as long-term natural host tissue without pathologic changes for the viruses identified by PCR techniques, perhaps except parvovirus B19. To find out cases with mild or moderate signs of inflammation and where a search for virus genome and/or genetic disorders can lead to further knowledge, our suggested immunohistochemical criteria (2) may be helpful.

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Response

To the Editor: Dettmeyer *et al.* acknowledge that our results did not confirm those of their studies and suggest they may be a result of different methods. They used MAb to CD45RO to identify T lymphocytes in the myocardium in their study (1); however, this antibody may identify macrophages and Langerhans cells (2); other studies have also shown a lack of specificity for T lymphocytes (3). CD45RO lymphocytes, the memory T lymphocytes, are a subset of the total T-lymphocyte population that includes CD45RA (or naïve) T lymphocytes. In

contrast, we used MAb against CD3, which identify all T lymphocytes. Dettmeyer *et al.* searched for viral genome in myocardial samples fixed for <48 h in neutral-buffered formalin; these samples could be expected to potentially yield more viruses than ours, which had been embedded in paraffin for months to years after buffered formalin fixation (4). They also searched for a larger number of viruses than we did. Both of these factors may help explain the different rates of viral detection in the two studies.

Whether the criteria for myocarditis used by Dettmeyer *et al.* are more rigorous than those used for adults is perhaps moot given the sudden infant death syndrome (SIDS) and control cases in both their and our studies were infants. More importantly, however, the minimal level of myocardial inflammation necessary to cause sudden infant death has yet to be established in humans and in animal models. That is why we chose to simply semiquantitatively assess the number of myocardial T lymphocytes and macrophages per unit area rather than use another's or our own arbitrary baseline cell number to define myocarditis, thereby speculating that it is sufficient to cause sudden infant death. Nevertheless, this will remain a very complicated and complex issue because less inflammation located directly in the conduction system is presumably required to cause a lethal arrhythmia than the amount of inflammation located elsewhere in the myocardium. In this regard, we certainly agree with Dettmeyer *et al.* that multiple sections of myocardium should be examined in all cases of sudden infant death in which a cause of death is not apparent from the death scene or the gross autopsy examination.

Given differing results from our studies, we agree that further research into the role of myocardial inflammation and viral agents, possibly interacting with underlying cardiac genetic disorders, in causing sudden infant death is warranted.

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