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LETTER TO THE EDITORS

Gamma-delta T cell expansion is closely associated with cytomegalovirus infection in all solid organ transplant recipients

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We read with interest the manuscript published by Puig-Pey *et al.* entitled ''Characterisation of gamma-delta T cells in organ transplantation'' [1]. For a better reading of this paper, we would like to add several comments on the basis of our experience in γδ T cell immunomonitoring in kidney transplant recipients (KTR), to enlighten their role in the context of cytomegalovirus (CMV) infection. Indeed, in KTR, we demonstrated, 10 years ago, that CMV infection is the sole parameter independently associated with a persistent

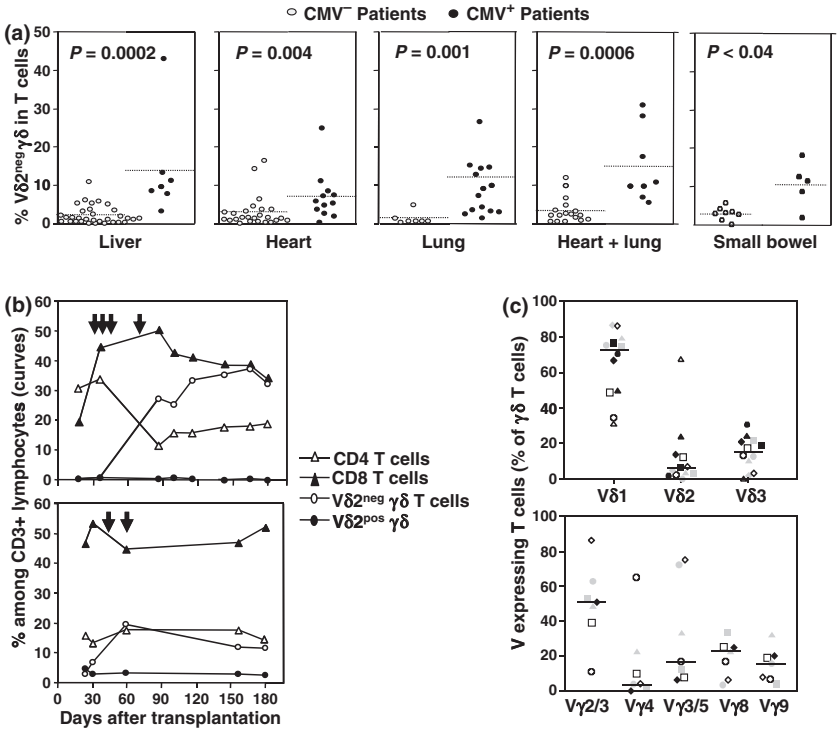

Figure 1 (a) High percentages of Vδ2^{neg} γδ T cells are associated with CMV infection in the heart, liver, lung, lung + heart and small bowel transplant recipients. The percentage of Vδ2^{neg} γδ T cells in the peripheral blood of liver, heart, lung, heart + lung and small bowel transplant recipients was determined by flow cytometry and compared between patients who suffered from CMV infection post-transplantation (CMV$^+$ patients, full circles) and those who did not (CMV$^-$ patients, empty circles). Dashed lines represent the mean value for each group of patients. The statistical differences between groups of CMV$^-$ and CMV$^+$ donors were tested using the unpaired Mann–Whitney *U*-test. (b) Vδ2^{neg} γδ T cell expansion occurs during CMV infection in transplant patients. The course of CD4+, CD8+, Vδ2^{neg} γδ T cells and Vδ2^{pos} γδ T cell subsets was longitudinally followed post-transplantation and are represented in one liver-recipient (upper panel) and in one lung-recipient (lower panel) suffering from CMV infection, as detected by pp65 antigenemia or CMV DNA in bronchoalveolar liquid (black arrows), respectively. It is noteworthy that Vδ2^{neg} γδ T cell expansion was followed in both patients by the resolution of CMV infection. γδ T cell percentages remained stable at least 6 months post-transplantation. (c) Combinatorial diversity of γδ T cells in CMV$^+$ lung- and/or heart-transplanted patients. The Vδ1,2,3 T-cell receptor variable region expression by γδ T cells was analyzed by flow cytometry in three lung, one heart and eight lung + heart recipients (upper panel), and Vγ2,3,4,5,8,9 expression was analyzed in seven of these recipients (lower panel). Analysis was carried out in whole blood by triple labeling with anti-Vδ or γ, anti-panδ and anti-CD3 monoclonal antibodies. γδ mainly expressed the Vδ1 and Vδ3 variable regions of the TCR.

expansion of circulating γδ T cells [2]. This expansion is associated with the resolution of the infection, and late γδ T cell expanders suffer from longer infections, suggesting an anti-viral role for these cells *in vivo* [3]. Since then, CMV-associated expansion of γδ T cells has also been observed by other groups in immunodeficient children [4,5], neonates [6], stem cell transplant recipients [7], and healthy volunteers [8].

As confirmed by Dr Sanchez-Fueyo's group, we have demonstrated that only Vδ2^{neg} γδ T cells (including Vδ1 and Vδ3 T cells) are involved in this stable expansion overtime [9], and that they display a highly biased repertoire suggestive of antigen-driven selection or CMV-mediated amplification of an initially restricted Vδ2^{neg} γδ T cell population [9]. The Vδ2^{neg} γδ T cells expand concomitantly with CMV-specific CD8+ T cells in the blood of KTR and share a similar effector/memory T_{EMRA} phenotype [8,10]. *In vitro,* they display T-cell receptor-dependent cytotoxicity against CMV-infected cells and inhibit CMV multiplication through interferon-γ production [11].

Puig-Pey *et al.* show that HCV infection is also associated with an increase in the Vδ1/Vδ2 ratio, but the significance is low ($P = 0.046$). Moreover, they do not mention whether a significant increase in Vδ1 γδ T cells is observed in HCV-infected liver transplant recipients (LTR). This is an important issue because in our previous study in KTR, the infections with other viruses than CMV (including HCV) were conversely associated with a reduced number of γδ T cells. If this decrease concerns only Vδ2 T cells, this could explain the increase in the Vδ1/Vδ2 ratio without the need for a rise in Vδ1 number. Consequently, we think that further investigations such as multivariate analysis are mandatory to confirm a relationship between HCV and Vδ2^{neg} γδ T cell expansion.

Previous studies by Dr Sanchez-Fueyo's group have concluded that Vδ1 γδ T cell expansions in LTR are associated with operational tolerance [12,13]. However, in their present paper, they demonstrate that quantification of Vδ1 γδ T cells does not allow for accurate discrimination between tolerant LTR and recipients requiring maintenance immunosuppression. On the contrary, they confirmed that ''CMV infection constitutes the main force shaping the repertoire of peripheral blood Vδ1 γδ T cells'' in LTR and KTR. We have also shown that CMV-mediated expansion of Vδ2^{neg} γδ T cells is a general phenomenon observed in all CMV-infected solid-organ transplant recipients (Fig. 1). The percentage of Vδ1 γδ T cells they found in CMV-negative LTR is equivalent to that of healthy individuals (compare figure 4 and figure 1 of their paper). This result confirms that ''transplantation procedure per se'' or ''the chronic exposure to pharmacological IS'' cannot induce Vδ2^{neg} γδ T cells expansion in the absence of CMV infection. The high prevalence of CMV infection in the LTR cohort of Dr Sanchez-Fueyo's group (90%) may explain why they missed the critical link between this virus and Vδ1 γδ T cell expansion previously.

As Vδ2^{neg} γδ T cell expansion is a cell signature of CMV infection, monitoring of these cells after transplantation holds promising interest. Moreover, their ability to kill both CMV-infected cells and carcinoma cells *in vitro* [11], and their association with reduced cancer risk in CMV-infected KTR [14], make their close monitoring of particular relevance to post-transplant outcomes.

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Conflict of interest

The authors have no conflicting financial interests. There is no study sponsor.

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