Methods

Determination of lymphocyte subsets by flow cytometry

For determination of T cell subsets within the $CD4^+$ and $CD8^+$ cells, 100 µl of whole blood collected with EDTA was stained during 15 min at room temperature with the corresponding mAb. The following fluorochrome-labeled mAbs were used: PerCP/Cy5.5-anti-CD4 (SK3), APC-H7-anti-CD8 (SK1), FITC-anti-CD45RA (L48), V450-anti-CD27 (MT271), PE-anti-HLA-DR (TU36), and BV510-anti-PD1 (EH12.1). After red blood cells lysis using FACS Lysing Solution (BD) for 7 min, cells were washed twice with PBS/BSA and acquired. After gating on $CD3^+$ cells, subsets within the $CD4^+$ and $CD8^+$ cells were defined as naïve T cells ($CD45RA^+CD27^+$), central memory T cells (T_{CM}, $CD45RA^-CD27^+$) and effector memory T cells (T_{EM}, $CD45RA^-CD27^-$). Activated $CD4^+$ and $CD8^+$ T cells were characterized as $HLA\text{-}DR^+$ cells and exhausted T cells were defined as $PD1^+$ cells. Cells were acquired in a FACSCanto II flow cytometer (BD) and analyzed using the FlowJo software v10.0.7 (Treestar, Inc.).