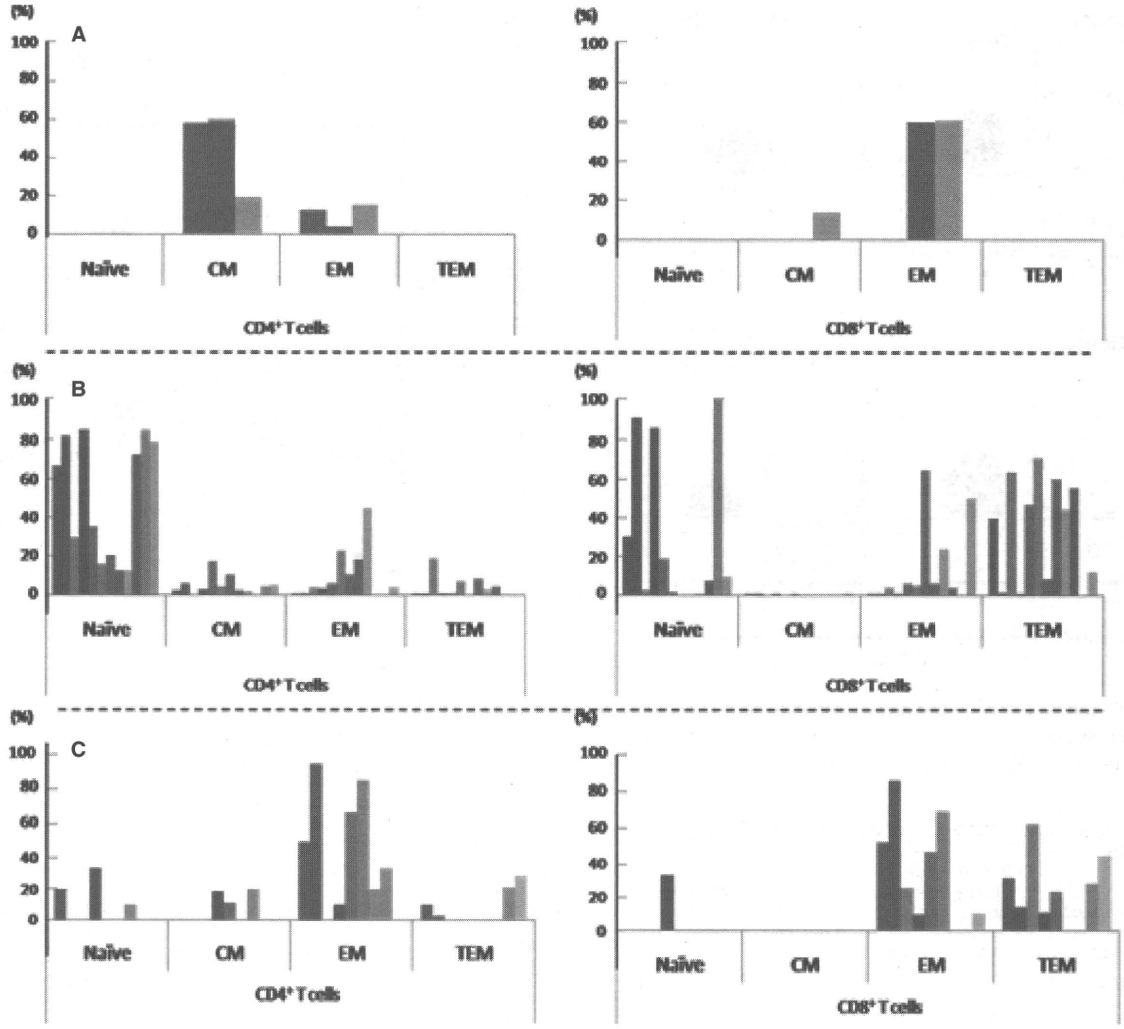

Figure 3 Phenotypic patterns of GPI-AP$^-$ T cells in different patient groups. The percentages of four different T-cell subsets defined by the expression of CD45RA, CCR7 and CD62L in CD4$^+$ and CD8$^+$ GPI-AP$^-$ T cells are shown. (A) Alemtuzumab-treated patients (n = 3); (B) bone marrow failure patients showing GPI-AP$^-$ cells in all lineages of blood cells (n = 12); (C) PNH-T$^+$ patients (n = 9). CM, central memory cells; EM, effector memory cells; TEM, terminal effector memory cells; PNH, paroxysmal nocturnal hemoglobinuria; GPI-AP, glycosylphosphatidylinositol-anchored protein.

cells in 12.8% of patients with various type of BMF. Although the percentage of GPI-AP$^-$ T cells in these patients was very low, such an increase in GPI-AP$^-$ T cells was undetectable in 57 healthy individuals and they persisted more than 2 months. The presence of GPI-AP$^-$ T cells was originally interpreted to indicate the ability of *PIGA* mutant HSC in the BMF patients to differentiate into multi-lineage blood cells (18–21). However, the GPI-AP$^-$ cells were undetectable in any other lineages of cells other than T cells in PNH-T$^+$ patients whose clinical features were similar to those of other BMF patients with GPI-AP$^-$ myeloid cells. The presence of such PNH-T$^+$ patients within the population of patients with immune-mediated BMF cannot be explained by the escape of GPI-AP$^-$ cells from T-cell attack against T-cell precursors, because T-cell precursors are not the specific target of the immune attack in patients with BMF.

The presence of PNH-T$^+$ patients can be explained by several mechanisms. One possibility is that the CD48$^-$CD59$^-$ T cells are remnants of GPI-AP$^-$ cells that used to be present in other lineages of cells. A previous study showed GPI-AP$^-$ T cells to persist in patients who underwent remission of PNH probably due to their longevity (33). The patients with long-standing disease like patients 3, 10, and 12 may have possessed small populations of GPI-AP$^-$ cells in the myeloid cells after the disease onset and lost all but the T cells with time.

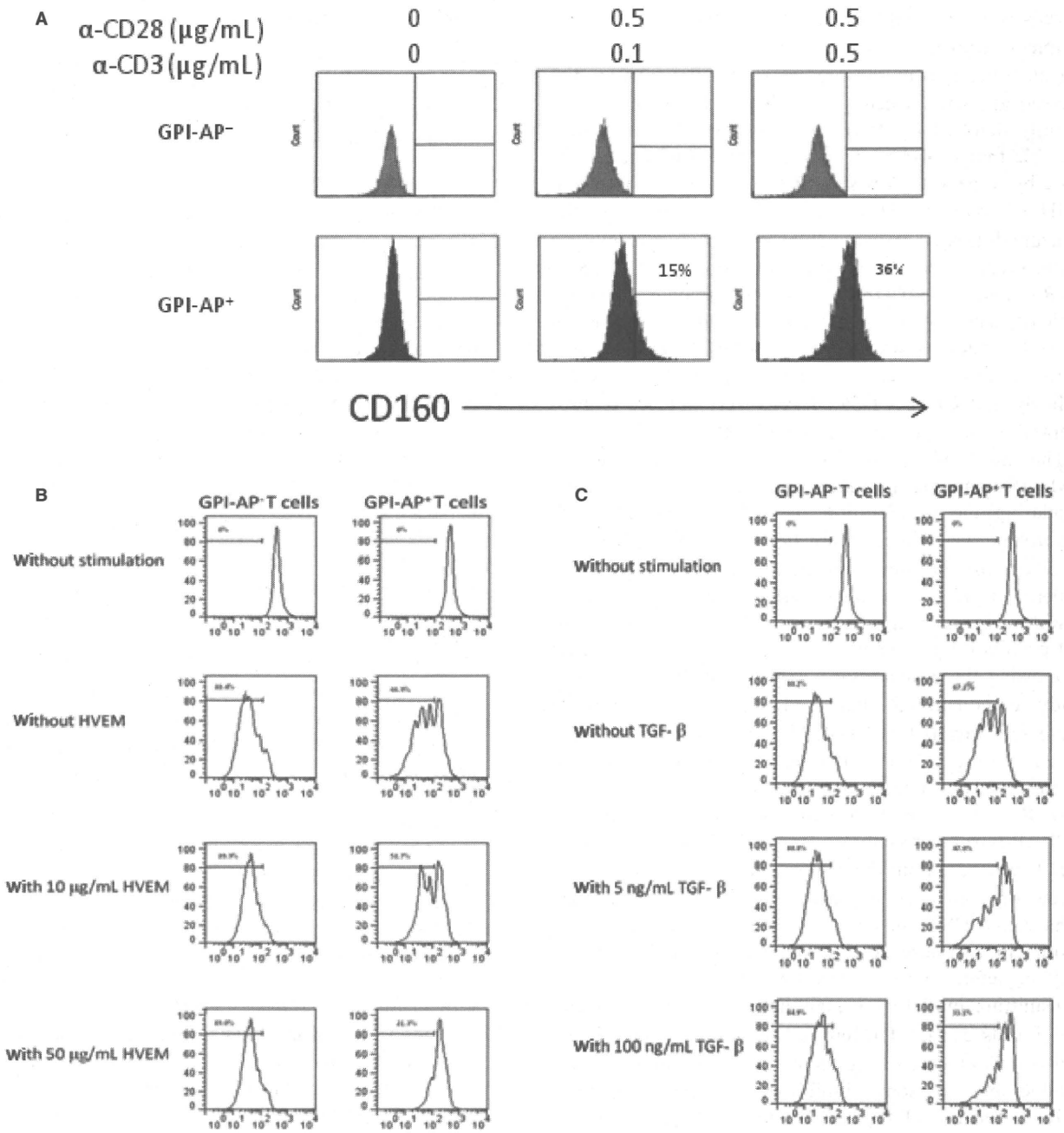

Figure 4 The effects of HVEM and TGF-β on the proliferation of GPI-AP$^+$ and GPI-AP$^-$ T cells induced by anti-CD3 and anti-CD28 mAb stimulation. PB CD3$^+$ T cells from three bone marrow failure patients were cultured in the presence of anti-CD3 and anti-CD28 mAbs for 10 d with or without HVEM and TGF-β. (A) CD160 expression by GPI-AP$^+$ T cells induced by anti-CD3 and anti-CD28 mAb stimulation compared with GPI-AP$^-$ T cells. The numbers show the percentage of CD160$^+$ cells. T-cell proliferation in the presence of different concentrations of HVEM (B) or TGF-β (C) was assessed using the carboxyfluorescein diacetate succinimidyl diester assay. The figures show representative results from one patient. The numbers denote the percentage of cells which underwent cell division. PB, peripheral blood; HVEM, herpesvirus entry mediator; GPI-AP, glycosylphosphatidylinositol-anchored protein.

However, this mechanism cannot account for PNH-T$^+$ patients in which the disease persisted for <1 yr. Another possibility is that mechanisms other than immune-mediated attack against HSCs confer proliferative advantage to GPI-AP$^-$ T-cell precursors or memory T cells. The treatment of patients with lymphoid malignancies or allogeneic stem cell transplant recipients with anti-CD52 mAb allows proliferation of GPI-AP$^-$ T cells that existed in the patients or BM donors before treatment (34, 35). Indeed, donor-derived CD48$^-$CD59$^-$

T cells were detectable in all three stem cell transplant recipients who received a conditioning regimen containing alemtuzumab in the present study. Previous studies showed auto-Abs specific to DRS-1 and moesin are frequently detected in PNH^+ patients (36, 37). It is thus possible that $GPI\text{-}AP^-$ T cells may be induced to proliferate by some auto-Abs specific to GPI-APs on T cells in $PNH\text{-}T^+$ patients. However, $GPI\text{-}AP^-$ T cells in alemtuzumab-treated patients showed a distinct phenotype pattern characterized by the expression of CD45RA, CCR7, and CD62L from that detectable in $PNH\text{-}T^+$ patients. There was no apparent T lymphocytopenia in $PNH\text{-}T^+$ patients which should occur in patients possessing auto-Abs specific to T cell antigens. It is therefore unlikely that $CD48^-CD59^-$ T cells were induced to proliferate by auto-Abs specific to GPI-APs.

The most likely explanation for the presence of $PNH\text{-}T^+$ patients is that humoral factors negatively regulating the proliferation of both HSCs and T-cell precursors via their interaction with GPI-APs are involved in the development of BMF in $PNH\text{-}T^+$ patients. Cytokine-mediated selection of *PIGA* mutant HSCs has been proposed as a mechanism for preferential proliferation of $GPI\text{-}AP^-$ cells (38), but no evidence supporting this mechanism has been shown. The present study demonstrated that $GPI\text{-}AP^-$ T cells show a decreased sensitivity to HVEM that transmit inhibitory signals through a GPI-AP receptor CD160 (27), as well as to TGF-β, a well-known inhibitor of haematopoiesis (39). Recent studies have demonstrated the presence of GPI-AP-type co-receptors for TGF-β (40). Although the T cells used in the current study were not T-cell precursors, memory T cells in the PB T cells may behave like HSCs in terms of their dormancy and activation in response to appropriate stimulation. HSCs may be rendered to express some GPI-APs capable of transmitting inhibitory signals upon activation as memory T cells express CD160 and as a result, both HSCs and T-cell precursors or memory T cells may become invulnerable to some inhibitory cytokines, such as TGF-β, because of the lack of GPI-AP type-receptors. Further analyses of T cells may therefore be useful for identifying GPI-AP type TGF-β receptors which permit the preferential proliferation of HSCs with *PIGA* mutation in patients with BMF.

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Disclosure of conflicts of interest

All authors have no financial or personal relationships with other people or organizations that could inappropriately influence this study. The authors declare no competing financial interest.

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