Epstein-Barr virus–specific T cell and NK cell responses in paediatric patients with infectious mononucleosis or post-transplant lymphoproliferative disorder: abridged secondary publication

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KEY MESSAGES

1. In patients with post-transplant lymphoproliferative disorder (PTLD), Epstein-Barr virus (EBV)-specific $CD8^+$ polyfunctional T cells were limited at 24 months after diagnosis, despite clinical remission.
2. In patients with PTLD, the frequency of potent degranulating $CD56^{dim}NKG2A^+KIR^-$ NK cells diminished from diagnosis to clinical remission.
3. In patients with PTLD, plasma EBV viral loads were weakly correlated with blood tacrolimus levels. Prolonged immunosuppression may result in suboptimal immune control of EBV.

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Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a rare but potentially life-threatening complication, frequently associated with Epstein-Barr virus (EBV) among transplant recipients who receive immunosuppressants to suppress T-cell function. Natural killer (NK) cells have an important role in controlling viral infection. This study aimed to explore the roles of distinct NK cell subsets and EBV-specific T cells in the control of EBV infection among patients with infectious mononucleosis (IM) or PTLD who were receiving tacrolimus. The effects of tacrolimus on the development of T and NK cells and the control of EBV loads were also determined.

Methods

We recruited 20 children with IM and 15 children with PTLD. Diagnosis of IM was based on clinical symptoms (including fever, cervical lymphadenopathy, pharyngitis, and hepatosplenomegaly) and the serological pattern of primary EBV infection. Diagnosis of PTLD was based on histological features of biopsy tissues (n=14) or positron emission tomography–computed tomography findings (n=1).

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised blood. Development of T and NK cells was determined by flow cytometric analysis. NK cell functions were assessed through expression of the proliferation marker Ki67 and via co-culture of PBMCs with the cell lines LCL721.221 or K562. EBV copy numbers in plasma and PBMCs were quantified. Correlations between EBV copy numbers and tacrolimus levels were determined using a two-tailed Spearman test.

Results and discussion

We assessed the development of EBV-specific polyfunctional T cell (PFC) and NK cell subsets. In patients with IM or PTLD, EBV-specific $CD4^+$ and $CD8^+$ T cells were identified at diagnosis and at post-diagnosis time points after EBV peptide stimulation. Among patients with PTLD, frequencies of BMLF1-specific $CD8^+$ T cells significantly increased at 24 months after diagnosis, and an increasing trend of EBV-specific $CD8^+$ T cells over time was observed, suggesting restoration of T-cell function after recovery. Among patients with IM, we observed a decreasing trend of EBV lytic antigen BMLF1- and BZLF1-specific $CD4^+$ PFCs but an increasing trend of EBV latent antigen EBNA1-specific $CD4^+$ PFCs at 12 months after diagnosis. Among patients with PTLD, a significant decrease in EBV lytic antigen BZLF1-specific $CD4^+$ PFCs was identified at 24 months after diagnosis. Among patients with IM—but not those with PTLD—we observed a decreasing trend in the frequency of EBV lytic antigen BMLF1-specific $CD8^+$ PFCs but an increasing trend of EBV latent antigen EBNA1- and EBNA3A-specific $CD4^+$ PFCs at 12 months after diagnosis. Additionally, the frequencies of all EBV lytic and latent antigen-specific $CD8^+$ PFCs remained low at 24 months after

diagnosis. Among patients with IM, $CD4^+$ and $CD8^+$ PFCs showed an increase in functionality over time. However, among patients with PTLD, EBNA3A-specific $CD8^+$ PFCs were only able to gain two additional combinations of function after recovery. This lack of polyfunctional EBV-specific $CD4^+$ and $CD8^+$ T cells suggested impairment of T-cell function due to long-term immunosuppressive drug therapy.

We assessed the frequencies of different NK cell subsets from diagnosis to 12 or 24 months. Most patients with IM or PTLD showed >90% $CD56^{dim}$ NK cells within their $CD3^-CD56^+$ NK cell population in peripheral blood. We dissected the distinct NK cell subsets within $CD56^{dim}$ NK cells. Among patients with IM, $NKG2A^+KIR^-$ NK cells constituted the majority of $CD56^{dim}$ NK cells, whereas among patients with PTLD, $NKG2A^-KIR^+$ NK cells were predominant. Longitudinal analysis of subset frequencies showed significant differences between the two cohorts. In patients with PTLD, the low frequencies of $NKG2A^+KIR^-$ NK cells were not related to treatment, and the difference in frequency of the $CD56^{dim}NKG2A^+KIR^-$ NK cell subset suggested suboptimal development of NK cells.

We found significantly higher frequencies of Ki67-expressing $CD56^{dim}NKG2A^+KIR^-$ NK cells in patients with IM than in patients with PTLD from diagnosis to 3 months after diagnosis. In contrast, the level of Ki67-expressing cells among $CD56^{dim}NKG2A^-KIR^+$ NK cells did not significantly differ between the two cohorts, nor did it change over time. A prior study demonstrated the importance of the $CD56^{dim}NKG2A^+KIR^-$ subset in the immune control of EBV.[1] The $CD56^{dim}NKG2A^+KIR^-$ NK cell subset showed more degranulation than $CD56^{dim}NKG2A^-KIR^+$ NK cell subset. Interestingly, patients with IM or PTLD showed similar degranulation levels of the $CD56^{dim}NKG2A^+KIR^-$ NK cell subset in response to LCL721.221. In patients with PTLD, the $CD56^{dim}NKG2A^+KIR^-$ NK cell subset could degranulate efficiently upon stimulation with EBV-infected B cells. The diminished frequency of potent degranulating $CD56^{dim}NKG2A^-KIR^+$ NK cells might lead to suboptimal immune control.

Plasma and PBMC EBV viral levels were significantly higher in patients with PTLD than in patients with IM at diagnosis and at 12 months post-diagnosis (data not shown). Despite a significant decrease in plasma viral loads over time, some patients with PTLD (n=4) showed persistently elevated EBV viral loads up to 24 months. All patients with PTLD showed persistently elevated EBV viral loads in their PBMCs up to 24 months (data not shown). We assessed the kinetics of plasma EBV viral loads and trough blood tacrolimus levels in 15 patients with PTLD from diagnosis onwards. Patients with increased blood tacrolimus levels had increased plasma EBV viral loads (data not shown). Consistent with these findings, plasma EBV viral loads showed a weak correlation with blood tacrolimus levels (r=0.1620, P=0.0191). Prolonged immunosuppression might play a role in suboptimal immune control of EBV, as indicated by persistent EBV viraemia and impaired T- and NK-cell development in patients with PTLD.

The new knowledge generated by this research study can be used to design specific immune assays that complement measurement of EBV loads in the management of PTLD. The findings also may lead to the development of a clinical algorithm to guide reduction of immunosuppression or pre-emptive treatment of PTLD.

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Disclosure

The results of this research have been previously published in:

1. Lam JKP, Azzi T, Hui KF, et al. Co-infection of cytomegalovirus and Epstein-Barr virus diminishes the frequency of $CD56^{dim}NKG2A^+KIR^-$ NK Cells and contributes to suboptimal control of EBV in immunosuppressed children with post-transplant lymphoproliferative disorder. Front Immunol 2020;11:1231.

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