BK virus (BKV) is a ubiquitous polyomavirus, which persists within the CD4+ T cells after primary infection. In immunocompromised patients, BKV reactivation may occur, which can result in, e.g., hemorrhagic cysts after allograft stem cell transplantation or loss of the allograft after renal transplantation. The detailed characterization of BKV-specific T cells is the basis for a better understanding of the BKV immune response. This in turn provides the foundation for the development of strategies towards the prevention or treatment of infections in immunocompromised patients, for example, the adoptive transfer of virus-specific T cells.

**Results**

**BKV-specific T cells are rare in healthy donors**

PBMC from six randomly selected healthy donors were stimulated with various peptide pools for immunodominant antigens of BKV, JCV, CMV, influenza, EBV, and AdV, and for positive control with a mixture of CMV/EBV/influenza PMHC class I–restricted peptides. After 6 hours, IFN-γ production within the CD4+ and CD8+ T cell compartments was analyzed by intracellular staining using the Rapid Cytokine Inspector. CMV-, EBV-, and AdV-specific IFN-γ+ T cells were clearly detectable in several donor samples. In contrast, IFN-γ+ BKV- and JCV-specific T cells were detectable only at very low frequencies, between 0.01 and 0.03%, in five out of six donor samples.

**Procedure for the rapid antigen-reactive T cell enrichment (Rapid ARTE)**

Recently, a protocol for the sensitive functional analysis of up to 2.5×10⁷ PBMC from six randomly selected healthy donors was developed. This so-called antigen-reactive T cell enrichment (ARTE) protocol allows the stimulation of up to 2.5×10⁷ PBMC. After six hours cells are fluorescently labeled for CD154 and intraacellular staining. The rapid ARTE protocol for enumeration of BKV-specific T cells was adapted. Up to 2.5×10⁷ PBMC from 14 healthy donors were left untreated or stimulated with BKV/peptide pools covering the complete sequence of the large T antigen (LT) and virus protein VP1. Both proteins are immunodominant targets for T cell immunity. After six hours, cells were treated as described in figure 2, and the absolute numbers of enriched CD1954+ CD4+ T cells were determined. In blood samples of each donor, BKV-reactive CD4+ T cells were found. Absolute numbers ranged between 97 and 2340 per 1×10⁷ PBMC.

**Conclusion**

- We developed a rapid (7 h) and convenient protocol to detect and characterize very rare antigen-specific CD4+ T cell subpopulations in blood samples.
- The process does not involve any centrifugation step and requires only minimal hands-on time.
- High-resolution characterization of rare T cell subsets is achieved combining magnetic cell enrichment and multiparameter flow cytometry analysis of CD1954+ T cells.
- In contrast to conventional flow cytometry analysis, this approach enabled us to identify BKV-specific T cells and specify their function.