In times of limited material, expansion of T cells in vitro has become a standard practice across many clinical and basic immunological procedures; such as haematopoietic stem cell transplantation, the study of biopsy specimens, immunomonitoring of clinical trial patients and the study of samples from neonates and children. Classically, mitogenic lectins, such as phytohaemagglutinin (PHA-P) or the phorbol ester phorbol 12-myristate 13-acetate, have been used for initiating T cell expansion. However, a more physiologically relevant method of T cell stimulation mimics the interaction with antigen-presenting cells through the use of anti-CD3 and anti-CD28 monoclonal antibodies (mAb) (Martin et al, 1986). This procedure has been improved in recent years, by fusing anti-CD3 and anti-CD28 mAb to microbead structures (Pene et al, 2003). Here, the initial signal is provided by CD3 molecule activation and the co-stimulatory signal is provided by CD28 molecule activation. In this study, we assessed whether human T-activator CD3/CD28 Dynabeads or PHA-P could amplify T cells from human umbilical cord blood (UCB) or adult peripheral blood mononuclear cells (PBMC) without distorting the baseline ex vivo T cell repertoire.

We first set out to establish the expansion potential of human T-activator CD3/CD28 Dynabeads versus PHA-P. T cell cultures were initiated by adding either PHA-P or Dynabeads to PBMC or UCB cells. Total cell numbers for PHA-P-stimulated cultures, CD3/CD28 Dynabead-stimulated cultures and unstimulated T cell cultures were recorded on days seven and 14 (Fig. 1A). Both PHA-P and Dynabeads were equally potent in expanding T cells. Interestingly, at day 14, Dynabeads were slightly more efficient at expanding adult PBMC, and PHA-P was slightly more efficient at expanding UCB. In donor C18S, from a starting population of only 2.8 × 10⁶ cells, the Dynabeads yielded 5.95 × 10⁶ cells in 14 d; an ~21-fold increase in cell number. An identical fold increase was also observed in the second UCB sample (UC2) (data not shown). Interestingly, Dynabead stimulation was better able to preserve the ex vivo CD4:CD8 ratio compared with PHA-P stimulation (Fig. 1B). This repertoire stability was also evident when examining epitope-specific T cell frequency, where RAKFKQLL/B8-specific populations remained clearly detectable and stable for the duration of culture (Fig. 1C). In contrast, over time, PHA-P cultures exhibited a tendency to drift towards a CD8⁺ rich state in both the UCB and adult PBMC samples (Fig. 1B). The frequencies of RAK/B8-specific populations were also more variable and more difficult to detect following PHA-P stimulation (Fig. 1C), indicating some preferential CD8⁺ clonotypic outgrowth of the PHA-P-stimulated lines.

We next sought to investigate T-cell receptor (TCR) dynamics during amplification through a temporal and quantitative dissection of TRBV gene usage. Using the same samples employed for the growth kinetics analysis, we dissected the total TCR repertoire through co-labelling CD8⁺ and CD4⁺ T cells with one of a panel of 25 TRBV-specific mAb, and examined the repertoires by flow cytometry. TRBV repertoire analysis of UCB T cell cultures from donor UC5, and adult PBMC cultures from donor C18S, is shown in Fig. 2. In cultures from all three donors, the TRBV repertoires were proportionally amplified using either stimulus (data for UC2 not shown). The TRBV repertoire of CD4⁺ T cells within the cultures was especially well conserved.

Apart from a handful of epitopes from herpesviruses and HIV, the vast majority of T cell responses against pathogenic and self targets are difficult to detect with current means. Thus, in vitro T cell culture is often used to amplify lymphocyte numbers to a level where the identification of epitope-specific T cells is achievable without donor exsanguination. However, in vitro amplification has the potential to artificially warp the TCR repertoire. The total TCR repertoire dissection discussed above suggested both Dynabead and PHA-P stimulation kept the repertoire broadly intact during expansion. However, we sought evidence that stimulation did not drastically distort individual epitope-specific repertoires. Here, we employed the model RAK/B8-specific response in adult donor C18S. During expansion, variation of the repertoire was observed over time (Fig. 2C), although these variations were much less pronounced in Dynabead-stimulated cultures than in PHA-P-stimulated cultures. The TRBV repertoire of PHA-P-stimulated RAK/B8-specific T cells showed substantial perturbation at day seven, which became more noticeable as time in culture progressed, with very strong skewing toward TRBV7-1 by day 14. Conversely, the day 14 Dynabead culture showed minimal distortion.

It is possible that various modifications to the protocol could be made to further augment T cell expansion, including optimization of the Dynabead:T cell ratio (Trickett & Kwan, 2003) or enrichment of CD3⁺ cells or depletion of CD25⁺ regulatory T cells prior to Dynabead addition. Indeed, CD25⁺ depletion has recently been shown to increase T cell expansion.
How these protocol modifications affect TCR repertoire dynamics is unknown. UCB T cell expansion is of particular interest in light of new methods in the identification and quantification of naïve epitope-specific T cell precursors (Moon et al., 2007). However, initial murine estimates indicate that the frequency of these precursors is low, requiring large numbers of lymphocytes for analysis. Indeed, in humans, and in particular UCB, the ability to examine more than a single epitope remains a challenge. This method allows for the theoretical generation of more than one billion T cells from a single UCB sample, allowing for simultaneous examination of many different naïve epitope-specific repertoires from a newborn.

In this study, we have investigated whether human T-Activator CD3/CD28 Dynabeads can effectively expand T cell repertoires from UCB and adult PBMC in a proportional manner. Using this culture method, very small starting numbers of T cells were expanded to large numbers within 14 d. When comparing with baseline PBMC, the CD4:CD8 ratio was maintained and both the epitope-specific and total TCR repertoires remained largely unperturbed. Conversely, while PHA-P stimulation also expanded lymphocytes very efficiently, the compound distorted both the CD4:CD8 ratio and the epitope-specific repertoire. In summary, the T cell amplification method described here provides a simple, effective approach to expanding T cells from limited starting material, in a proportional manner across both naïve and antigen-experienced populations.

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Author contributions

MAN performed data generation. MAN, AKS, SRB and JJM contributed to the design and interpretation of data, project management, and writing of the manuscript.

Competing interests

The authors declare no competing interests.

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References


