Introduction

T cell immunotherapy often requires expansion of a small selected starting population in vitro. To achieve therapeutic doses, this population is required to undergo multiple and rapid rounds of replication. Rapid T cell expansion raises the possibility of inducing senescence or an aged phenotype, both of which are detrimental to the recipient patient and thus it is important to understand aging characteristics of T cells that have undergone this process. The WAVE Bioreactor system is often the equipment of choice for the final expansion phase of the T cell therapy manufacturing process before patient infusion.

Here we present data from an investigation of the aging phenotype in T cells selectively expanded from peripheral mononuclear cells (PBMCs) using the WAVE Bioreactor 2/10 system. T lymphocytes that have become senescent can be characterized by phenotypic changes. CD57, a marker frequently used in T cell phenotyping, becomes up-regulated as cells senesce. Other markers that can be used to study aging of T cells include CD45RA and CD27. Additionally, phosphorylated H2AX, a histone marker for double-stranded DNA breaks, accumulates in the nuclei of senescent cells. Our data show that T cells expanded using the WAVE Bioreactor 2/10 system retain a healthy phenotype with no indication of emerging senescent phenotype.2,3

Material and Methods

PBMCs from four healthy donors, separated by Ficoll-Paque™ Premium density gradient media (GE Healthcare Life Sciences), were counted and characterized by flow cytometry. The number of T lymphocytes (CD3+ cells) was determined and Dynabeads™ CD3/CD28 Expand beads (Invitrogen, Life Technologies) were added at a ratio 3:1 (beads:T cell). Cells were cultured at 0.5 – 1 × 10^6 cells/mL in X-VIVO™ 10 (Lonza supplemented with 5% human AB serum off the clot (PAA Laboratories), 10 ng/mL IL-2 (Peprotech), 2 mM stable glutamine and 1X Pen/Strep solution (PAA Laboratories) at 37°C in 5% CO2. At day 5 cells were transferred (in approximately 700 mL at a density of 0.5 – 10^6 cells/mL) to a perfusion Cellbag™ 2 L bioreactor on a WAVE Bioreactor 2/10 system. In the initial phase of expansion, the cell density was maintained at 0.5 × 10^6 cells/mL by adding fresh culture medium until day 7 had been reached. At this stage, perfusion was started and carried out as shown in the protocol (Fig 1). An overview of the expansion process in the WAVE Bioreactor 2/10 system is shown (Fig 1). Phenotypic analysis of cells (including CD4, CD8, and CD3) and markers associated with senescence in T lymphocytes (CD57, CD45RA, CD27, and H2AX) was performed by flow cytometry at days 0, 5, 10, and 14.

Results

T cells were expanded using the WAVE Bioreactor 2/10 system and characterized for markers related to T cell aging. Results show that T cells retain a healthy phenotype after rapid expansion and do not express markers of senescence. Figure 2 shows that senescence markers are down-regulated in expanded CD8+ T cells.

Furthermore, we studied expression of CD57 and H2AX, both associated with senescent cells. CD4+ T cells show an up-regulation of CD57 at day 14, whereas CD8+ T cells show a down-regulation (Fig 3). T cells, both CD4+ and CD8+, expanded with the WAVE Bioreactor 2/10 system do not show an escalation in DNA damage during the culture (Fig 4).

Conclusions

T cells expanded with the WAVE Bioreactor 2/10 system do not express markers of senescence nor show signs of DNA damage. The data show a selection of highly differentiated CD8+ T cells at the end of the expansion period. We conclude that the WAVE Bioreactor 2/10 system is an effective mechanism for the rapid expansion of T cells without compromising cell health.

References