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T cells expanded in the WAVE Bioreactor™ 2/10 system maintain a healthy phenotype

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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[1]. 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 Expander 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 37ºC in 5% CO_2. 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 1 L 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.

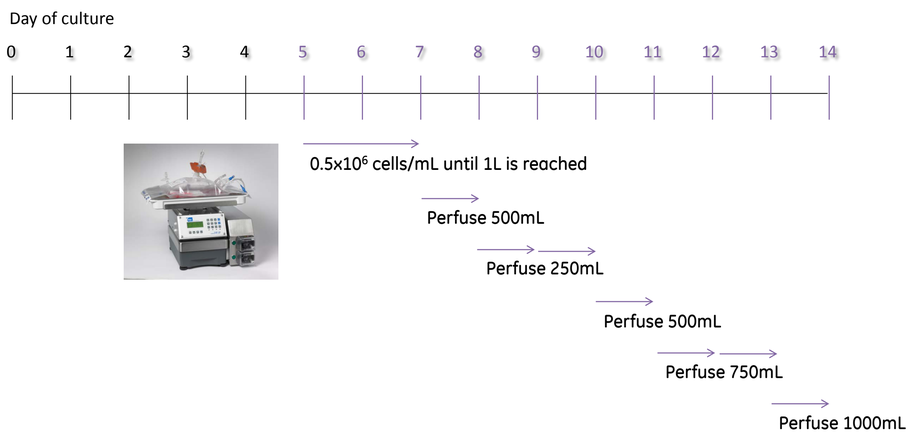

Fig 1. Overview of the expansion process in WAVE Bioreactor 2/10 system. Numbers indicated in black correspond to culture in static and numbers in lilac to expansion with the WAVE Bioreactor 2/10 system. Cells were inoculated in a perfusion Cellbag 2 L bioreactor at day 5 to a density of 0.5×10^6 cells/mL. Media was added to maintain the cell density until the final culture volume of 1 L had been reached. Subsequently, perfusion was initiated, typically at day 7, at rates indicated in the figure. Perfusion rates imply the amount of media exchanged in the Cellbag bioreactor in mL/24 h.

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.

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.

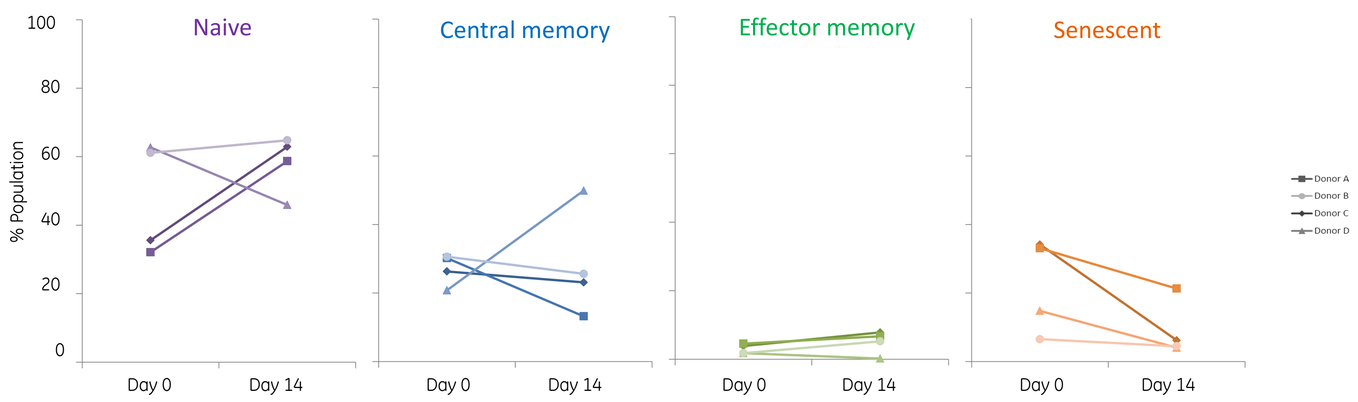

Fig 2. Loss of senescent $CD8^+$ T cells during culture. Aging of $CD8^+$ T cells was investigated by measuring expression of CD45RA, CD27, and/or CD28 at initiation and termination of expansion for each donor. Data show that $CD8^+$ effector memory cells are increased by the end of expansion. Contrarily, a decline of $CD8^+$ T cells expressing senescence markers is seen at day 14 compared to day 0. Naïve cells are $CD45RA^+$ and $CD27^+$. Central memory cells are $CD45RA^-$ and $CD27^+$. Effector memory cells are $CD45RA^-$ and $CD27^-$. Senescent cells are $CD45RA^+$ and $CD27^-/CD28^-$.

Furthermore, we studied expression of CD57 and H2AX, both associated with senescent cells. $CD4^+$ T cells shows an up-regulation of CD57 at day 14, whereas $CD8^+$ T cells shows 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).

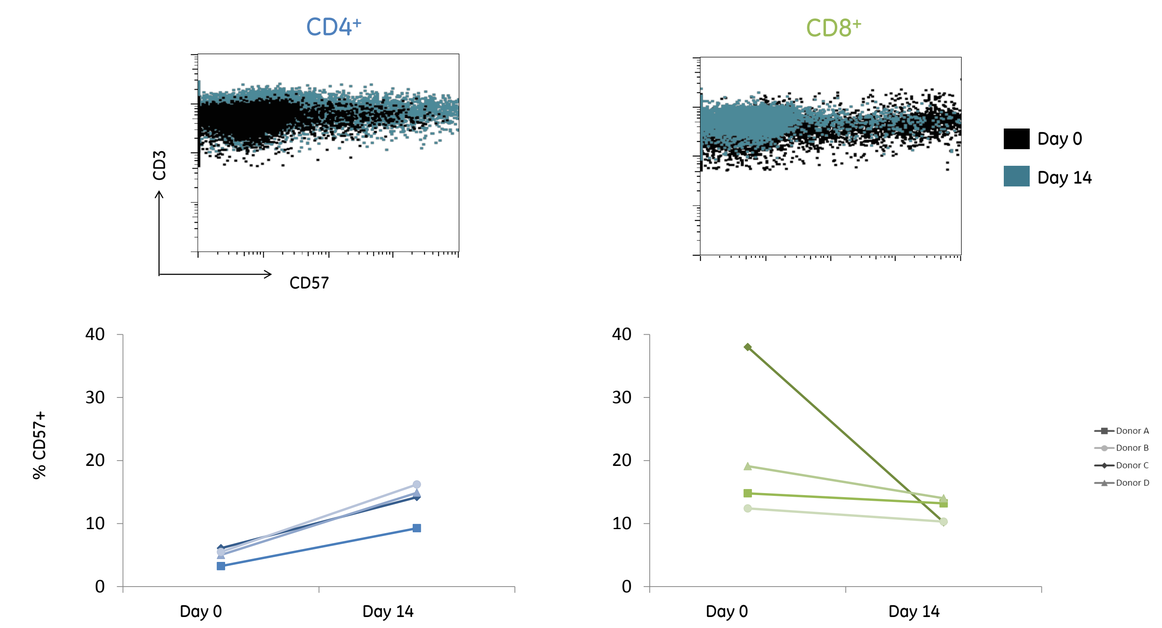

Fig 3. Marker for replicative senescence is down-regulated in $CD8^+$ T cells. CD57 expression on $CD4^+$ and $CD8^+$ T cells to the left and right, respectively. $CD8^+$ T cells have a down-regulation of CD57 expression at day 14, which implies that they have a healthy phenotype at the end of expansion.

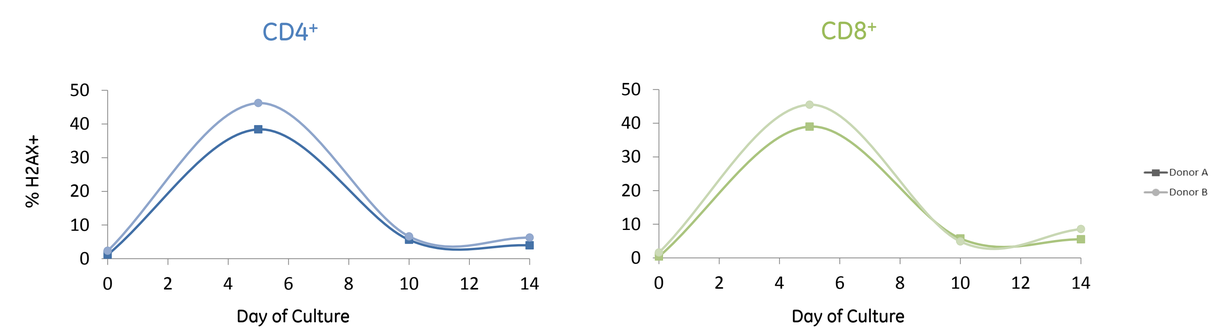

Fig 4. DNA damage is not cumulative in T cells. Plots show the percentage of phosphorylated H2AX in $CD4^+$ and $CD8^+$ T lymphocytes to the left and right, respectively. Indicating that DNA damage, a sign of senescing cells, is not increased following expansion.

References

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