Rapid Expansion of Functional Human T Cells Using a Novel Serum-Free and Xeno-Free Culture Medium

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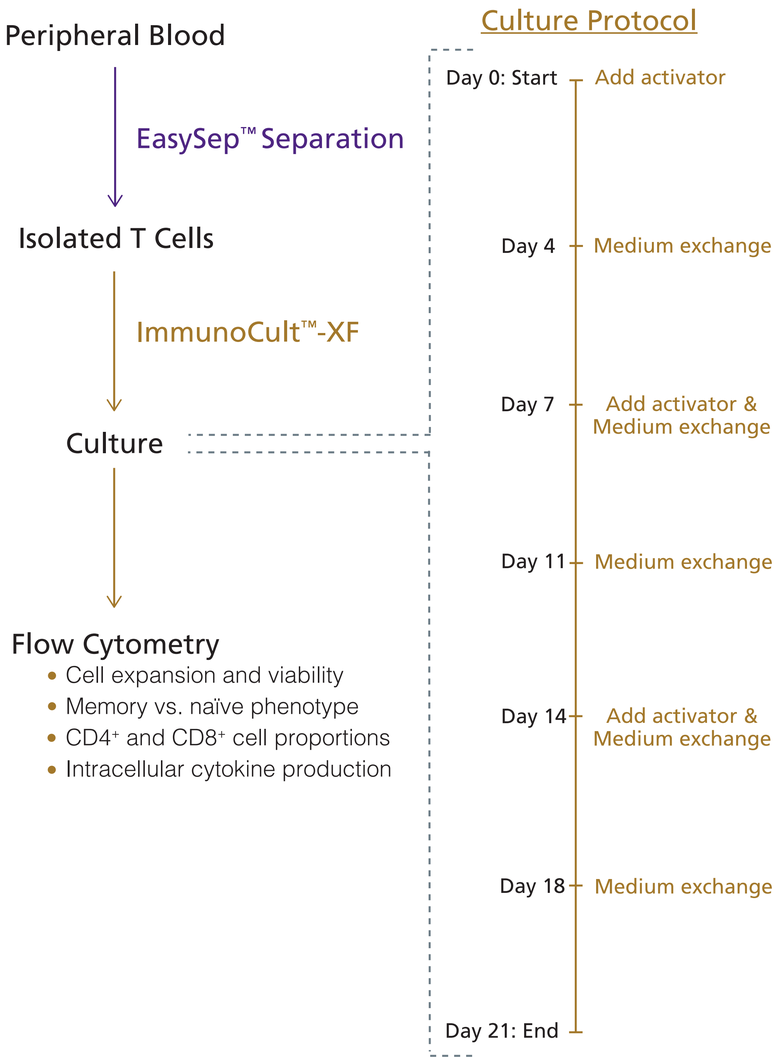
Background

The adoptive transfer of functionally active, genetically modified T cells encoding receptors for tumor antigens is a promising treatment strategy in cancer immunotherapy. To produce an adequate number of these T cells for therapeutic efficacy, expansion *in vitro* is necessary. Traditionally, T cells are activated and expanded in media that contain human serum to promote cell growth and viability. However, because serum contains many uncharacterized components and possible infectious agents, a defined, serum-free medium is preferable. To address these problems, we have developed a novel serum-free and xeno-free medium called ImmunoCult™-XF T Cell Expansion Medium that supports the rapid expansion of activated T cells.

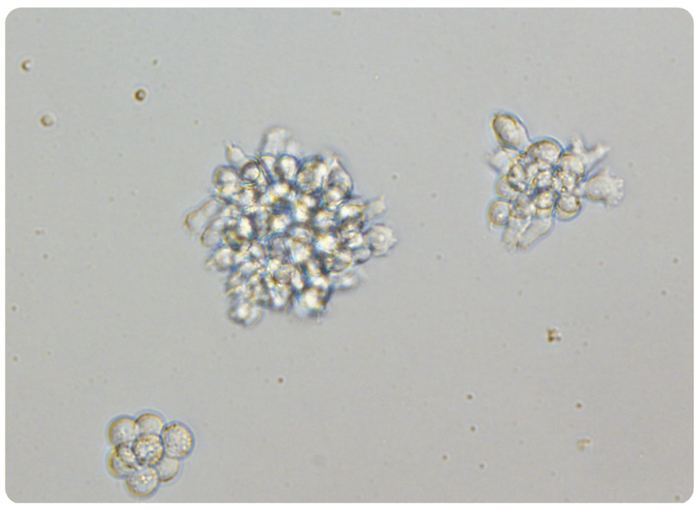
Methods

Human T cells were isolated from peripheral blood by negative selection using the EasySep™ Human T Cell Enrichment Kit ($CD3^+$ purity: >95%). The isolated T cells were seeded into a 96-well plate at 50,000 cells/mL, activated using the soluble ImmunoCult™ Human T Cell CD3/CD28/CD2 Activator, and expanded in ImmunoCult™-XF T Cell Expansion Medium supplemented with 10 ng/mL of recombinant human IL-2 for 21 days. T cells were re-activated during the course of expansion every 6 - 8 days.



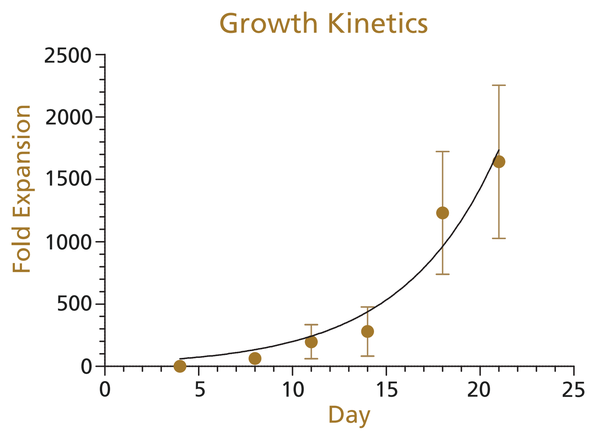
Results

FIGURE 1: T cells expand in morphologically typical clusters in ImmunoCult™-XF T Cell Expansion Medium



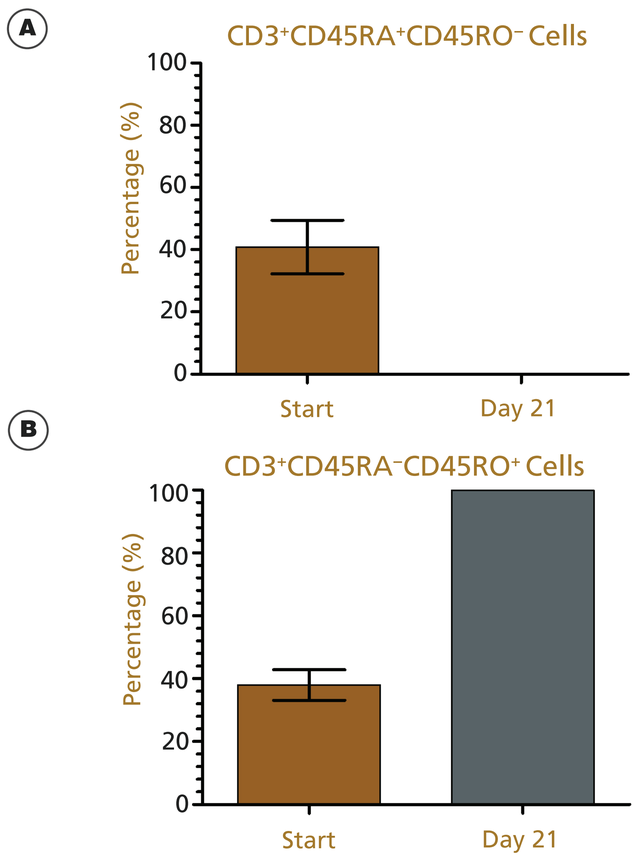
T cells were seeded at 5,000 cells per well (or 50,000 cells/mL) into a 96-well plate with ImmunoCult™-XF T Cell Expansion Medium and activated with ImmunoCult™ Human T Cell CD3/CD28/CD2 Activator at the start of the culture. Clusters of cells were observed in culture after 4 days, indicating cell activation and expansion. The bright field image was taken using a 20X objective on a microscope.

FIGURE 2: ImmunoCult™-XF T Cell Expansion Medium supports over 1,000-fold T cell expansion during 3 weeks of culture



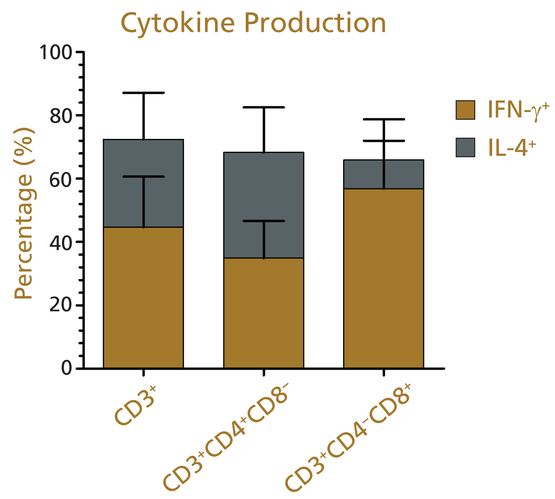
T cells were analyzed using flow cytometry on days 4, 8, 11, 14, 18, and 21. Fold expansion was normalized to the initial cell seeding density of 5,000 cells per well. The viability of the T cells was 85 ± 5% (mean ± S.D.; n = 6; data points with error bars represent mean ± S.D; solid line represents the best-fitting curve for exponential cell growth determined using non-linear regression analysis).

FIGURE 3: T cells adopt a memory phenotype after activation and expansion in ImmunoCult™-XF T Cell Expansion Medium



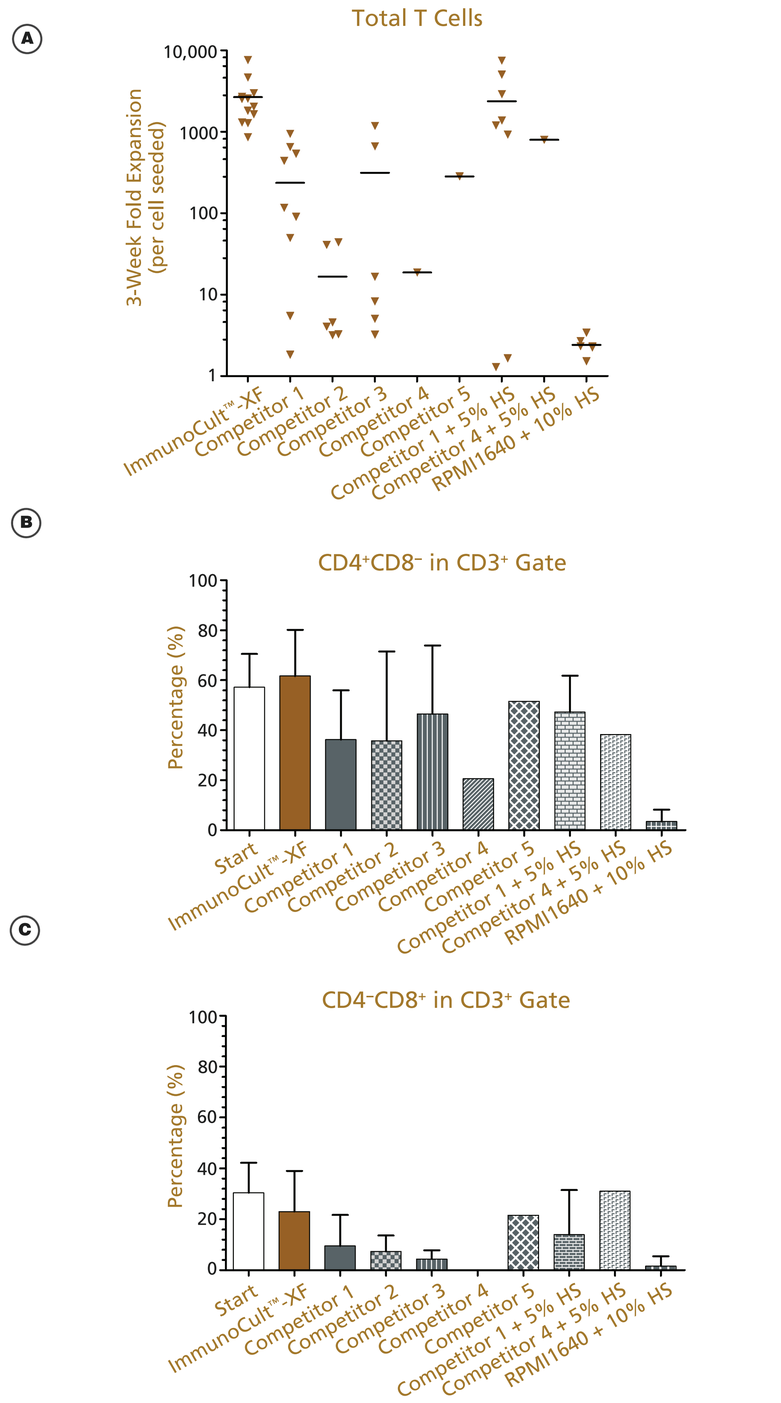
The phenotypes of the T cells at the start of culture and after 21-day expansion in ImmunoCult™-XF T Cell Expansion Medium were examined by flow cytometry. At the start of culture, the T cells were on average **A)** 41 ± 9% (mean ± S.D.) $CD3^+CD45RA^+CD45RO^-$ naïve T cells and B) 38 ± 5% $CD3^+CD45RA^-CD45RO^+$ memory T cells. **B)** After activation and expansion, essentially all of the T cells were $CD3^+CD45RA^-CD45RO^+$, indicating a memory T cell phenotype (n = 4; columns with error bars represent mean ± S.D.).

FIGURE 4: T cells expanded in ImmunoCult™-XF T Cell Expansion Medium produce IFN-γ and IL-4



After 21 days of culture, T cells were analyzed by flow cytometry for intracellular IFN-γ and IL-4 after stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 4 hours at 37°C. The percentage of intracellular IFN-γ^+ or IL-4^+ cells in the $CD3^+$, $CD3^+CD4^+CD8^-$, and $CD3^+CD4^-CD8^+$ cell subsets were determined. After expansion in ImmunoCult-XF T Cell Expansion Medium for 21 days, the expanded T cells produced both IFN-γ and IL-4, with 45 ± 16% (mean ± S.D.) IFN-γ^+ and 28 ± 15% IL-4^+ cells in the $CD3^+$ total T cell population, 35 ± 12% IFN-γ^+ and 33 ± 14% IL-4^+ cells in the $CD3^+CD4^+CD8^-$ T cell subset, and 57 ± 15% IFN-γ^+ and 9 ± 13% IL-4^+ cells in the $CD3^+CD4^-CD8^+$ T cell subset (n = 6; columns with error bars represent mean ± S.D.).

FIGURE 5: ImmunoCult™-XF T Cell Expansion Medium supports higher levels of total T cell expansion than other serum-free and serum-supplemented media and maintains similar proportions of $CD4^+$ and $CD8^+$ cells as the start of culture



A) Total T cells were analyzed by flow cytometry after 21 days of culture for fold expansion, normalized to the initial cell seeding density of 5,000 cells per well. T cell expansion in ImmunoCult™-XF T Cell Expansion Medium was significantly better compared to T cell expansion in all other serum-free media tested and RPMI1640 + 10% FBS ($p < 0.01$, unpaired t-test). Only the addition of 5% human AB serum (HS) to some of the other media supported T cell expansion to similar levels as those obtained in ImmunoCult™-XF T Cell Expansion Medium (n = 1 - 12; each data point represents the mean of three replicate cultures per donor; horizontal line represents the mean of all donors). In addition, T cells were analyzed for the relative proportions of $CD4^+$ and $CD8^+$ cells after 21 days of culture. The percentages of **B)** $CD4^+CD8^-$ and **C)** $CD4^-CD8^+$ cells within the $CD3^+$ gate were determined by flow cytometry. At the start of the culture, 57 ± 13% (mean ± S.D.) of $CD3^+$ cells were $CD4^+CD8^-$ and 30 ± 12% were $CD4^-CD8^+$ (n = 17). After 21 days of culture in ImmunoCult™-XF T Cell Expansion Medium, the relative proportions of $CD4^+$ and $CD8^+$ cells were very similar to those at the start of culture, i.e., 62 ± 18% $CD3^+$ cells were $CD4^+CD8^-$ and 23 ± 16% $CD3^+$ cells were $CD4^-CD8^+$ (n = 12). Cultures in the other media typically resulted in lower frequencies of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells (n = 1 - 12; columns with error bars represent mean ± S.D.). Competitors 1 - 5 represent serum-free media from other suppliers. Competitors 1 and 4 were also tested after supplementation with 5% HS.

Summary

ImmunoCult™-XF T Cell Expansion Medium supports the activation of T cells by the soluble ImmunoCult™ Human T Cell CD3/CD28/CD2 Activator, and promotes robust expansion of human total T cells at levels similar to or exceeding those in other serum-free and serum-supplemented media. In addition, the expanded T cells have similar frequencies of $CD4^+$ and $CD8^+$ T cells as before culture, suggesting that the medium supports the expansion of both T cell subsets equally well. The expanded T cells are functional as demonstrated by their ability to produce IFN-γ and IL-4 cytokines upon stimulation by PMA and ionomycin. Taken together, when used with the soluble ImmunoCult™ Human T Cell CD3/CD28/CD2 Activator, ImmunoCult™-XF T Cell Expansion Medium provides a serum-free and xeno-free alternative to serum-containing media and bead-based activation reagents for research on the development of T cell-based immunotherapy for cancers and other disorders.