RAPID EXPANSION OF FUNCTIONAL HUMAN T CELLS USING A NOVEL SERUM-FREE AND XENO-FREE CULTURE MEDIUM

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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.

We have developed a novel serum-free and xeno-free T cell expansion medium called ImmunoCult-XF that allows for the rapid expansion of activated T cells. T cells ($5x10^4$ cells/mL) immunomagnetically isolated from peripheral blood were activated using a soluble CD2/CD3/CD28 activation reagent and expanded in ImmunoCult-XF supplemented with IL-2 (10 ng/mL) for 21 days, with re-activation every 6-8 days. The expansion of viable $CD3^+$ cells averaged 2,700-fold after 21 days in ImmunoCult-XF (n=12). This level of expansion was similar to that in X-VIVO 15 medium supplemented with 5% human serum (2,400-fold; n=8), and significantly higher than those in all other serum-free media tested (8-160 times higher; $p<0.05$; n=6). After activation and expansion, the T cells converted to a $CD45RA^-CD45RO^+$ memory phenotype (38±5% on day 0 versus 100±0.02% on day 21; n=4), while the frequencies of $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells remained relatively unchanged ($CD4^+CD8^-$: 57±13% on day 0 versus 62±18% on day 21; $CD4^-CD8^+$: 30±11% on day 0 versus 23±16% on day 21; n=11). Finally, the expanded T cells were functional as demonstrated by intracellular IL-4 and IFN-gamma expression (45±16% IFN-gamma$^+$ cells and 28±15% IL-4^+ cells; n=6) 4 hours after stimulation with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (1 μg/mL). Taken together, these results demonstrate that large numbers of functional T cells can be generated in vitro under a completely serum-free culture condition.