Comparison of Culture Media for In Vitro T Cell Expansion and Function

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Background
Identification of a reliable culture media to support in vitro T cell studies has become an important link in the chain of various immunology strategies. While many labs have chosen one favorite media for their T cell culture needs, it may be prudent to identify alternatives that can perform suitably, whether one works in the development of cell-based assays to screen potential drug candidates or generates and expands antigen-specific T cells. To address this issue, we have conducted a series of studies comparing the performance of several culture media.

Methods
A list of culture media (including several classic media + supplements as well as several new media) was compared to several commercially available T cell media in the generation of primary MLR (mixed lymphocyte reaction), antigen-recall assays (e.g., CMV, tetanus, antigen-specific T cell proliferation assay, as well as in anti-CD3/CD28-driven T cell expansion culture.

Cells
PBMC were obtained from normal donors in our study protocol. The protocol for collection of PBMC has been reviewed and approved by an accredited independent review board (IRB) and participants have given their informed consent for use of their cells in research. B-lymphoblastoid cells were inactivated by incubation with 50 μg/mL of mitomycin C at 37˚C. After this incubation, mitomycin C was removed by 3 washes with PBS. The inactivated cells were plated at 20,000 cells per well in a 1/2 bottom 96 well plate. Peptide antigen was added to indicated concentrations and tetanus toxin-specific T cells were added at 20,000 cells per well. The cultures were incubated for 4 days and proliferation was measured by addition of CellTiter Glo™.

Cytokine Analysis
Cytokines were analyzed using a U-Plex kit from Mesoscale Discovery. Interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) were routinely measured in the recall antigen assay.

Antigen-Specific Proliferation Assay
Tetanus toxoid-specific T cells were used to evaluate the effect of media formulations on antigen-specific proliferation. Antitoxin B-LCL were inactivated by incubation with 50 μg/mL of mitomycin C for 45 minutes at 37˚C. After this incubation, mitomycin C was removed by 3 washes with PBS. The inactivated cells were plated at 20,000 cells per well in a 1/2 bottom 96 well plate. Peptide antigen was added to indicated concentrations and tetanus toxin-specific T cells were added at 20,000 cells per well. The cultures were incubated for 4 days and proliferation was measured by addition of CellTiter Glo™.

Mixed Lymphocyte Reaction
B-lymphoblastoid cells were used to stimulate PBMC in a mixed lymphocyte reaction. The batch of PBMC and B-LCL were kept constant for this series of experiments. Proliferation of the B-LCL was prevented by incubation of the cells with 50 μg/mL of mitomycin C (Sigma) for 45 minutes at 37˚C. After this incubation, mitomycin C was removed by 3 washes with PBS and following the last wash the cells were suspended in the desired medium. The APC were added at a range of cell number per well in a 100 μL volume. PBMC were added at 20,000 cells per well in a volume of 100 μL of the appropriate medium. The culture was incubated for 5 days and proliferation was measured by addition of CellTiter Glo™.

Stimulation with Anti-CD3 and Anti-CD28
PBMC were stimulated with ImmunoCult™, an anti-CD3, anti-CD28 conjugate from Stem Cell Technology. This reagent was used at a range of concentrations in test media. PBMC were added at 1×10⁵ cells per well in 1/2 well plates or cultured in bulk for flow cytometric analysis.

Flow Cytometric Analysis
Fluorescent antibodies were purchased from Biologie and used at the volumes recommended by the manufacturer. Antibodies were incubated with cells for 15 minutes in the dark, washed once and data was acquired using a FACScan cytometer. Further analysis was performed using FCS Express (DeNovo Software).

Results
Classical media supplemented with several defined components can support primary in vitro responses as measured by cytokine production. Sustained T cell proliferation demands additional supplementation and revealed greater differences between media. One representative data from these studies is included in this abstract. This experiment demonstrates the effect of human AB serum (HS) and fetal bovine serum (FBS) added to the culture medium X-VIVO™ 15 (Lonza, Walkersville, MD). At low peptide concentrations (3 and 10 ng/mL), the presence of HS and FBS inhibits T cell proliferation compared to X-VIVO™ 15 alone.

Conclusions
• Supplementation of media with human serum does not support optimal cytokine production in the recall antigen assay.
• Minimal supplementation of X-VIVO™ 15 is sufficient to support cytokine production and proliferation in short-term (3-day) assays.
• Supplementation of culture medium with serum supports growth but requires higher concentrations of antigen.
• Choice of culture medium influences not only growth rate but phenotype and function of resulting T cells.