Retrovirology

Poster presentation

P16-33. Standardized serum-free cryomedia have minimum cytotoxicity and maintain antigen-specific T-cell response

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Background

The ability to analyze cryopreserved PBMC for antigen specific T-cells immunity is needed in evaluating response to immune based therapies. Comprehensive studies have demonstrated that the quality of frozen PBMC is critical and maintaining cell viability and functionality by using appropriate cryopreservation techniques is a key to the successful outcome of assays using PBMC. Different cryomedia additives impact the cell viability. The most common additive is FCS although it has been recognized that lots used for this purpose have to be chosen based on careful testing to avoid using serum that may lead to nonspecific stimulation of T-cells. In addition, the most widely used cryopreservation procedure for cells is based on adding DMSO. However, the amount of DMSO added must be reduced significantly due to its toxic impact on cells at room temperature. Therefore, we have developed novel freezing approaches aiming at the use of cryoprotectants having minimum cytotoxicity and maintaining T-cell functionality.

Methods

To measure the resulting PBMC recovery and viability, we have used trypan blue exclusion and FACS analysis by staining with propidium iodide as read-out systems. To measure T-cell functionality, we have used the interferon-γ ELISpot assay.

Results

We found that DMSO concentration can be reduced by addition of hydroxyethyl starch. We also tested different protein additives in comparison to the widely used FCS and found that bovine serum albumin (BSA) is an appropriate additive to substitute the potential immune modulating FCS. Using our new cryomedia, the PBMC recovery is more than 80% and the PBMC viability is more than 97%. Also the T-cell functionality measured by ELISpot is optimal with our new cryomedia.

Conclusion

Based on our experimental results, we could finally design two different, optimized cryomedia. Both media are standardized serum-free and manufactured under GMP conditions. In addition, one media contains a reduced amount of the DMSO.