PepSup™ - A Simple T-Cell Assay Providing the Highest Level of Standardization & Reproducibility

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Clinical immunotherapies targeting T-cells are used in an ever-increasing number of patients, in particular cancer patients. A reliable and economical way of monitoring the success of such therapies is a big challenge. Typically, antigen-specific T-cells are detected by ELISPOT or flow-cytometry (intracellular cytokines). However these methods are costly and difficult to standardize. PepSup™ is a simple, highly standardized, peptide-based cell stimulation assay designed to produce short-term culture supernatants from whole blood. These can be analyzed with respect to cytokines or other mediators of interest using ELISA or similar technologies that lend themselves to high levels of standardization. PepSup™ is an ideal tool for monitoring antigen-specific, stimulation-induced mediator secretion in a range of situations, including infection, transplantation, or tumor-specific immunity. Because of its high degree of standardization and hence reproducibility, it is particularly well suited to longitudinal patient monitoring. Sample processing only requires a programmable heat block and a simple bench-top centrifuge.

Introduction

The most frequently used antigen-specific tests for T-cell activation to date are the Elispot assay and intracellular cytokine detection by flow-cytometry (ICS). Before these technologies were available, proliferation and cytotoxicity tests (both very laborious but indirect) or ELISA (not as sensitive as today) were used to measure T-cell activation. In recent years a new generation of highly sensitive assays for the detection of secreted mediators has become available, some with the ability to multiplex analytes while using extremely small sample volumes. The advent of these tests has prompted us to devise a novel, highly standardized assay for T-cell activation based on peptide-induced mediator secretion in whole blood (WB). Stimulation is performed in ready-to-use tubes provided by JPT (**PepSup™ Cell Activation Kit**). This relieves the user of dissolving peptides in solvents like DMSO and subsequently media as well as aliquoting the dissolved peptides, which are both major sources of error and experimental variation in clinical studies. The only required experimental steps are adding 0.5 ml of WB to the PepSup™ tubes and incubating them in order to perform the assay (**Fig. 1**). The use of CO_2 independent, optimized media in the preparation of PepSup™ tubes has the advantage that they can be incubated with tightly closed lids in a programmable heat-block (no CO_2 incubator needed). Heat-blocks that can cool down samples to 4°C at the end of the desired incubation time will allow for delayed sample processing, for example, when incubating overnight.

Methods

The PepSup™ stimulation cocktail was prepared by dissolving PepMix™ peptide pools[1] in optimized PepSup™ stimulation media so that a concentration of 4 µg/ml of each peptide was achieved. Aliquots of 0.5 ml of the PepSup™ stimulation cocktail were then transferred into 2 ml reaction tubes. Tubes were closed and stored at -80°C until use.

WB from healthy volunteers was drawn into Na or Li-Heparin tubes. Prior to use, blood tubes were gently inverted several times to resuspend settled cells. Subsequently 0.5 ml of WB was pipetted into each sterile PepSup™ tube, gently washed into the PepSup™ stimulation cocktail. PepSup™ tubes were then closed, and incubated in a programmable heat block. After the desired incubation time (16h overnight) the block was cooled to 4°C. Samples were removed from the heat block in the morning and centrifuged at 1000 x g for 5 minutes. PepSup™ tubes were then opened without disturbing the pellet, and the supernatants carefully transferred to one or more previously labeled reaction tube(s) with a standard pipette. After processing all PepSup™ tubes in that manner, collected supernatants were stored at -80°C for subsequent analysis.

Secreted cytokines were analyzed using the MSD V-plex platform (Mesoscale Discovery, Rockville, MA, USA) and alternatively the Th1/Th2 cytometric bead array (CBA, Becton Dickinson, San Jose, USA). CBA samples were read on an Accuri C6 Cytometer (BD). Data analysis was performed according to the manufacturer's instructions.

For setting up the method, WB stimulation in a standard incubator with standard culture media (requiring CO_2) or in a heat block, with optimized PepSup™ media were compared side by side. Conditions were optimized so that at least the same stimulation result was obtained.

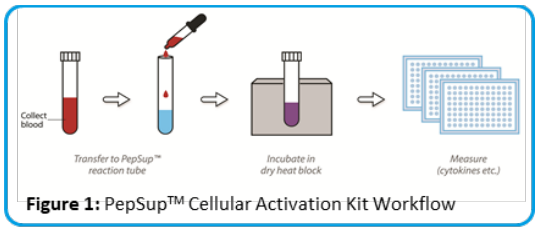

Figure 1: PepSup™ Cellular Activation Kit Workflow

Results

PepMix™ pools representing the CMV proteins, UL55 (gB), UL83 (pp65), UL122 (IE-2), and UL123 (IE-1)[2] showed efficient stimulation at the optimum concentrations of 2 µg/ml. This was also found for a range of other antigens (not shown).

We also tested PepSup™ stability over 6 months at -80°C using CMV UL83 and the new CEFX pool[3] (**Fig. 2**). A comparison of freshly prepared and stored PepSup™ tubes (-80°C, 6 months) showed no differences in the stimulation result, indicating that the ready-to-use reagents are stable. Additional stability testing (12 and 18 months) is ongoing.

Discussion & Conclusions

The novel PepSup™ format offered by JPT is an efficient way of measuring antigen-specific T-cell activation. It is, at the same time, easy to standardize and simple to use. JPT supports standardization by providing PepSup™ in a one-test-per-tube format. Compared to standard incubation in a humidified incubator with a 5% CO_2 atmosphere, the use of tightly closed tubes containing a CO_2-independent stimulation cocktail further minimizes user-dependent variation. Finally, standardizing the widely used antigen-specific T-cell assays based on intracellular cytokine detection (ICS, flow-cytometry) or Elispot has proven quite difficult as a result of longer, more complicated experimental protocols with far more scope for users to introduce unintentional variation, including the preparation of peripheral blood mononuclear cells. Measuring cytokines in the supernatant by a new generation, highly sensitive ELISA or another, equivalent platform provides a level of standardization that will be difficult to match by ICS or flow-cytometry.

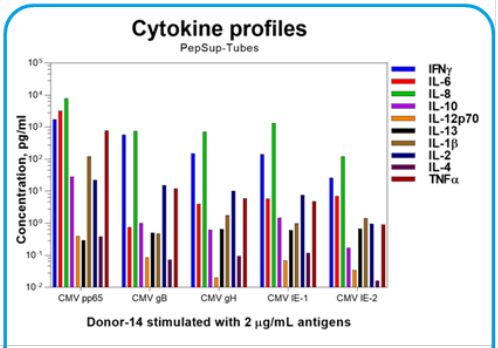

Figure 2: PepSup™ stimulation with a selection of antigens

The advantages of a standardized whole blood stimulation assay compared with the widespread procedures using PBMC were highlighted in a recent publication.[4] Whereas the reported assays used a standardized blood collection system and were not peptide-based, with respect to the laboratory handling procedures, JPT's PepSup™ format provides essentially the same technical advantages.

Thanks to the broad choice of PepMix™ reagents already available in JPT's catalog, T-cells specific to a wide range of antigens can be addressed immediately. With an ever-increasing number of T-cell-targeted biological therapies being tested in clinical trials, a simple and relatively inexpensive test format for monitoring individual therapeutic success is needed. PepSup™ is an ideal platform to fill this gap. Preliminary data suggests that PepSup™ is very sensitive even to small changes in antigen-specific T-cell numbers, and, in addition, can be used with multiple activation readouts.

References

[1]Maecker, et al., *J Immunol Methods* **2001**, *255*, 27-40.
[2]Bajwa et al., *The Journal of infectious diseases* **2016**.
[3]Castro et al., *The Journal of Immunology* **2018**, *200*, 120.115-120.115.
[4]Duffy et al., *Clin Immunol* **2017**, *183*, 325-335.

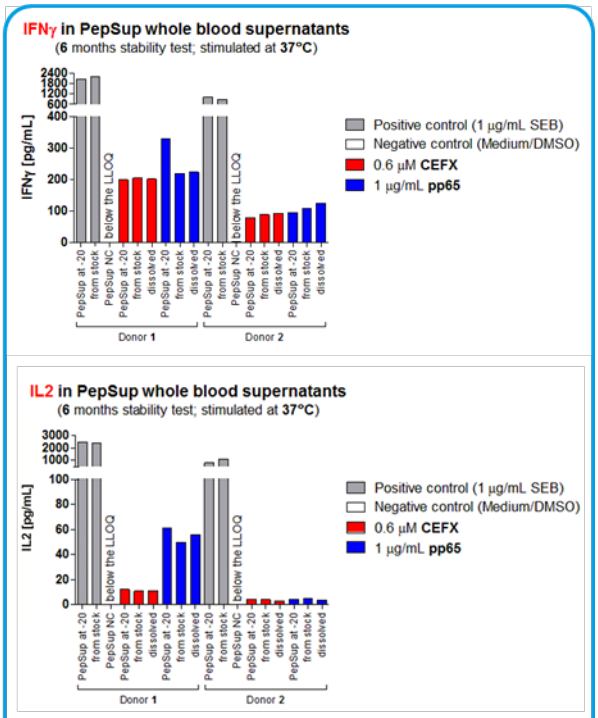

Figure 3: Results of stability test of PepSup™ tubes after 6 months storage

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The Company

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