INTRODUCTION

Cytokine Release Syndrome (CRS), also known as the “Cytokine Storm”, is a systemic inflammatory response characterized at the cellular level by the rapid release of excessive concentrations of cytokines (1,2). Patient symptoms range from mild fever to hypotension, multiorgan system failure, neurotoxicity and death. CRS is caused by a variety of events, including viral infection, antibody-based immunotherapy and cellular immunotherapy. In antibody-based immunotherapies, the incidence of CRS is relatively low, however, onset can occur within days and up to weeks of infusion in cellular immunotherapy. Evidence suggests that the risk and severity of CRS is influenced by the type of therapy, disease burden and patient characteristics, such as age. With respect to the latter, there is a higher incidence of CRS in pediatric patients. Other correlates include the “first dose effect”, i.e. the initial infusion stimulates a more robust response than subsequent infusions.

CRS severity is also modulated by the specific therapy administered (2). In cellular therapy, Chimeric Antigen Receptor (CAR) T constructs that include CD28 have been correlated with a higher risk for CRS than those that include 4-1BB. Other variables such as the robustness of T cell activation and expansion also correlate with CRS severity. Clinical studies (3) have correlated T cell expansion with increased Interferon Gamma (IFN-γ) release. Note that increased IFN-γ is also an early and prominent element of CRS. IFN-γ, along with IL-6, IL-10 and TNF-alpha are the core cytokines involved in CRS (2). IFN-γ activates macrophages which secrete excessive amounts of other cytokines such as IL-6, TNFα and IL-10. Lastly, it is important to keep in mind that IFN-γ is pleiotropic and has both harmful and beneficial effects. Although IFN-γ has a harmful role in CRS, it also been correlated with antiproliferative, proapoptotic and antitumor activities in the context of cancer (4).

We previously described the new 3rd generation R&D Systems® IFN-γ Quantikine® ELISA kit. Learn more about that assay here. Here, we describe the R&D Systems® Human IFN-γ Quantikine® High Sensitivity (HS) ELISA Kit, which has been designed with increased sensitivity for quantitation in serum and plasma. The minimal detectable dose (MDD) of this immunoassay is 0.173 pg/mL. Such sensitivity is particularly useful when assessing baseline IFN-γ secretion which has been reported to range from 0 to 20 pg/mL in healthy individuals (5-8).

METHOD

Two experiments were performed for this study. In the first experiment, serum and plasma were isolated from the whole blood of 30 healthy donors as described in (9). Serum was isolated using serum separating tubes (SST) containing clotting activating particles. Plasma was collected in EDTA containing tubes and Heparin containing tubes. IFN-γ was quantified in each sample using the R&D Systems® Human IFN-γ Quantikine® High Sensitivity (HS) ELISA Kit (Catalog # HSDIF0).

In the second experiment, serum samples were analyzed from three donors who received CAR T infusions. Ten time points were collected over 13 days, including pre and post-treatment. Investigators were blind to medical information from the donors. Samples were evaluated using 3 assays: the R&D Systems® Human IFN-γ Quantikine® High Sensitivity ELISA Kit, the Simple Plex IFN-γ Assay and the R&D Systems® Luminex® Human XL Cytokine Discovery Panel. Samples were analyzed according to instructions for each platform. Samples were evaluated at multiple dilutions in duplicate using the IFN-γ ELISA. Assay run time for each 4-analyte cartridge was approximately 1 hour. The concentrations of analytes in each sample were quantified by comparison to standard curves for each analyte, which were generated and pre-loaded onto each cartridge during manufacture. All data was obtained via triplicate results per biomarker per well.
RESULTS

Ninety serum and plasma samples from 30 apparently healthy donors were tested using the R&D Systems® Human IFN-γ Quantikine® High Sensitivity ELISA Kit. The minimal detectable dose (MDD) of this ELISA is 0.173 pg/mL. Of those samples, 23% were detected by the IFN-γ ELISA (FIGURE 1).

Next, we quantified IFN-γ in serum from 3 patients that were undergoing CAR T-cell therapy (FIGURE 2). Samples were collected before and after infusion. Although one patient exhibited high IFN-γ levels prior to the infusion, all patients demonstrated an increase in IFN-γ secretion after the infusion.

The IFN-γ secretion kinetics varied as a function of the individual. All patients’ IFN-γ serum levels were below 40 pg/mL by 5 days post-infusion.

Finally, given that Bio-Techne’s brands have many immunoassay solutions for measuring cytokines in serum, plasma, and cell culture supernates, we wanted to assess the degree to which data obtained with each platform are correlated. Serum samples described in FIGURE 2 were also quantified using the 3rd generation Simple Plex IFN-γ Assay and R&D Systems® Luminex® Human XL Cytokine Discovery Panel. Our data indicate that there is a high degree of correlation across all platforms (FIGURE 3).

CONCLUSIONS

CRS is a well-known consequence of viral infections such as influenza and COVID-19. It is also an adverse response to cell therapy. INF-γ secretion is an early and prominent feature of CRS that stimulates a myriad of downstream responses. Given its central role in CRS, sensitive and reliable assays for quantitating IFN-γ are crucial. Bio-Techne’s brands have over 30 years of experience developing and manufacturing assays for measuring cytokines. In this application note, we demonstrated that the new R&D Systems® Human IFN-γ Quantikine® High Sensitivity (HS) ELISA Kit quantifies IFN-γ secretion in serum and plasma to sub single digit pg/mL. Our data also indicate that the Quantikine HS ELISA is highly correlated with R&D Systems® Luminex® assays and Simple Plex assays. Given the high correlation coefficient, it is apparent that you can easily move from the Luminex assay to the Quantikine ELISA to the Simple Plex assay for routine measurements.
WHICH ASSAY SHOULD YOU USE?

We have previously demonstrated that the Quantikine ELISA and Simple Plex assays are great options for quantifying IFN-γ expression. Here, we demonstrate that the Quantikine HS assay is no different in the context of low level IFN-γ in serum or plasma. All platforms demonstrate consistency in measurement and high performance, making them ideal for your sample analysis. If you are familiar with running single-analyte ELISAs and wish to continue with plate-based ELISAs, then the Human IFN-γ Quantikine HS ELISA is a great option for serum or plasma samples. In contrast, the Simple Plex IFN-γ assay streamlines the process in situations where serum or plasma IFN-γ is measured frequently and routinely. This 90 minute automated assay reduces user error and is ideal for situations where comparisons across users and geographies is paramount. Finally, the Human XL Cytokine Luminex Performance Assay is ideal for those looking to measure up to 45 different cytokines simultaneously. This is quite useful for assessing the kinetics of IFN-γ, IL-6, IL-10 and TNF-α secretion in the context of viral infection or cell therapy administration.

REFERENCES

8. Yang, Y. et al. (2018) J of Transl Med. 16:165