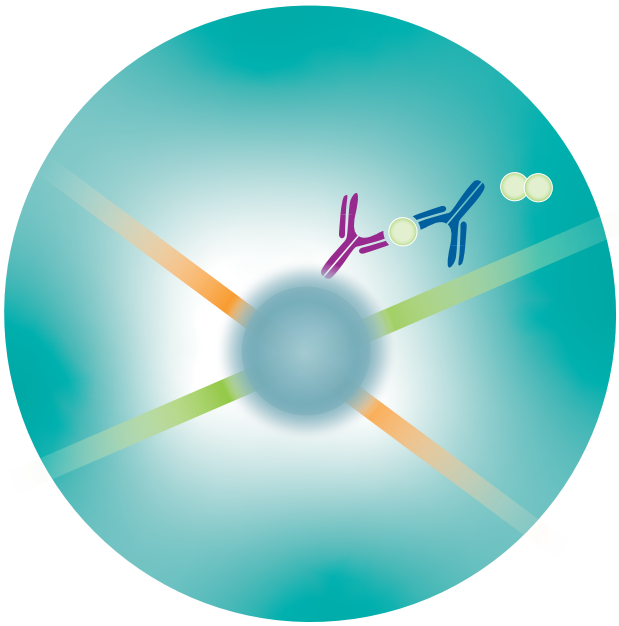
APPLICATION NOTE

Luminex® Multiplex Immunoassays for Classification of Th1, Th2, and Th17 CD4+ T Cell Differentiation

Developing CD4+ T cells in the thymus differentiate into various distinct subsets. Each subset produces a characteristic combination of cytokines to activate other immune cells, control ongoing immune responses, or manage immune memory.

Bio-Techne has developed R&D Systems® Luminex® Immunoassays to specifically quantify biomarkers of Th1/Th2 and Th9/Th17/Th22 differentiated CD4+ T cells. These two Luminex kits allow for the characterization of T cell lineages present in CD4+ T cells which have been induced towards specific T cell subsets.



METHODS

Cell Culture

Using the MagCellect™ Human CD4+T cell Isolation Kit (R&D Systems; Catalog # MAGH102), CD4+T cells were isolated from Human peripheral blood mononuclear cells cultured in RPMI and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. These cells were either untreated or treated with Th1, Th2 or Th17-inducing reagents.

Th1 Cells: Flasks were coated with 2 µg/mL goat anti-mouse IgG (AF007) overnight at room temperature. Wash 1X with PBS, and then coat with 1 µg/mL Ms x hCD3ε (MAB100) 2-3 hours at room temperature. Wash 1X with PBS prior to adding CD4+T cells seeded at 0.5 – 1 x 10^6/mL. To activate the cells the following polarizing cytokines and antibodies were then added to the flask and incubated for 5 days:

20 ng/ml rhIL-2 (202-IL)
40 ng/ml rhIL-12 (219-IL)
5 µg/ml Ms x hIL-4 (MAB304)
5 µg/ml Ms x hCD28 (MAB342)

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Further incubate stimulated cells for 24 hours with PMA (10 ng/mL; Tocris; Catalog # 1201/1) and Ionomycin calcium salt (500 ng/mL; Tocris; Catalog # 1704/1)

Th17 cells: Flasks were coated with 2 µg/mL goat anti-mouse IgG (AF007) overnight at room temperature. Wash 1X with PBS, and then coat with 1 µg/mL Ms x hCD3ε (MAB100) 2-3 hours at room temperature. Wash 1X with PBS prior to adding $CD4^+$T cells seeded at 0.5 – 1 x $10e^6$/mL. To activate the $CD4^+$ T cells the following cytokines and antibodies were then added to the flask and incubated for 5 days:

20 ng/mL rhIL-2 (202-IL)
20 ng/mL rhIL-23 (1290-IL)
10 ng/mL rhIL-1β (201-LB)
40 ng/mL rhIL-6 (206-IL)
5 µg/mL Ms x hCD28 (MAB342)

Further incubate stimulated cells for 24 hours with PMA (10 ng/mL; Tocris; Catalog # 1201/1) and Ionomycin calcium salt (500 ng/mL; Tocris; Catalog # 1704/1)

Th2 cells: Add $CD4^+$T cells seeded at 0.5 – 1 x $10e^6$/mL to an un-coated flask. To activate the $CD4^+$ T cells the following were then added to the flask and incubated for 5 days:

20 ng/ml rhIL-2 (202-IL)
5 ug/ml PHA

Further incubate stimulated cells for 24 hours with PMA (10 ng/mL; Tocris; Catalog # 1201/1) and Ionomycin calcium salt (500 ng/mL; Tocris; Catalog # 1704/1)

Luminex Fixed Panel Analysis

Cell culture supernates were analyzed with either the Luminex Th1/Th2 Fixed Panel (Catalog # LKTM008) or the Luminex Th9/Th17/Th22 Fixed Panel (Catalog # LKTM009). Samples were assayed according to the procedures outlined in the product inserts. Both kits are available from R&D Systems.

RESULTS

Cells polarized towards a Th1 phenotype secreted increased levels of IFN-α and TNF-β, as expected for Th1 differentiation (Figure **1B**). A hallmark of Th2 differentiation is increased levels of IL-4, IL-5, and IL-13, which we observed (Figure **1A**). Stimulating cells with IL-2, IL-23, IL-1β and IL-6 drove activated $CD4^+$ T cell differentiation to a Th17 phenotype, as observed by the secretion of IL-17A (Figure **1D**).

CONCLUSION

The cytokine profiles measured with our Luminex Fixed Panels for Th1/Th2 and Th9/Th17/Th22 were consistent with expected results for each respective T cell phenotype. R&D Systems fixed panels provide a convenient, efficient, and highly-validated immunoassay option to generate biomarker/cytokine profiles in response to intracellular and extracellular pathogenic bacteria, viruses, and fungi. These fixed panels are provided as an off-the-shelf product, making it easier and faster than ever to get the Luminex kits you need.

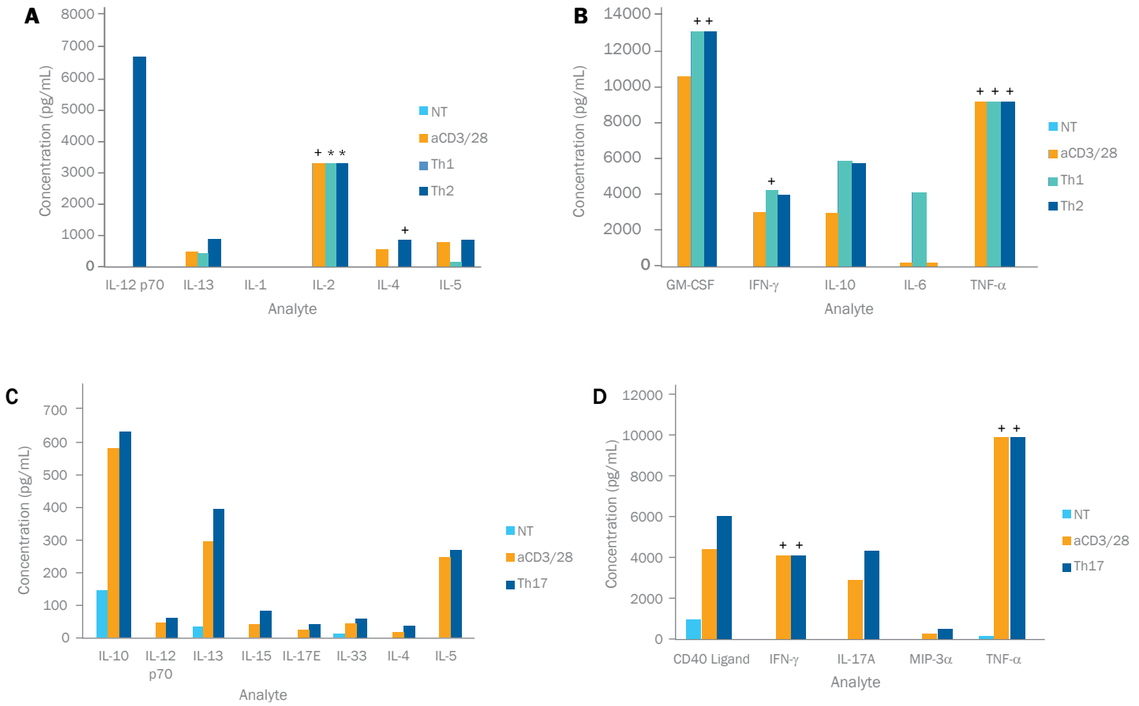

Figure 1 - Th1, Th2 and Th17 Cytokine Differentiation Profiles
A & B - Cytokine profile for the TH1/Th2 fixed panel analytes.
C & D - Cytokine profile for the Th9/Th17/Th22 fixed panel anayltes.
+ = values above the limits of the standard curve
• = stimulating cytokine
aCD3/28 = cells from an uncoated flask incubated in RPMI and 10% FBS for 5 days
NT = Non-treated cells from an antibody coated flask with only Ms x hCD28 added for 5 days
Note: The concentrations represented in this figure have not been corrected for the initial 1:2 sample dilution

REFERENCES

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