



Miltenyi Biotec

MACS
Quant X

High-throughput, multiplexed cytokine detection in mouse MLR

Pieter Kennis, Femke Calle and Laura Vannevel

Discovery – Ablynx (Sanofi)

Introduction

Therapeutics that target immune checkpoints have led to an immense breakthrough in the field of immuno-oncology. Analyzing immune cell function and interaction is among the most common assays for development of such therapeutics. Mixed lymphocyte reaction (MLR), for example, is commonly performed in pharmaceutical organizations to assess T cell responsiveness to mismatched major histocompatibility antigens (fig. 1). The ability to multiplex different analytes together with enhanced automation affords flow cytometry advantage over other traditional analytical tools for such complex assays.

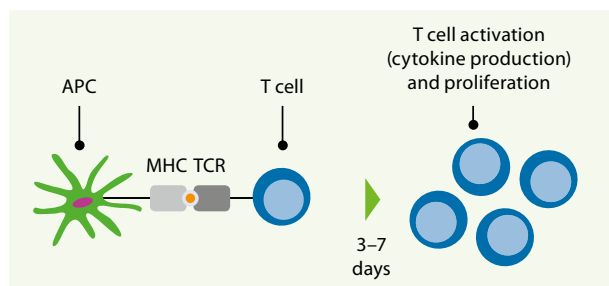


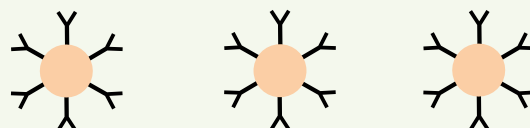
Figure 1: Mixed lymphocyte reaction resulting T in cell activation.

In this study, compounds targeting various receptors on myeloid and/or lymphoid cell compartments were evaluated in a mouse MLR. For some targets and target combinations, it is uncertain which cytokines will be produced after single or combination treatment. Therefore, several cytokines were evaluated using the MACSPlex Cytokine Kit from Miltenyi Biotec to find the best readout for the different treatments. Automated analysis and fast acquisition in a MACSQuant® X Flow Cytometer enabled increased throughput in the assay.

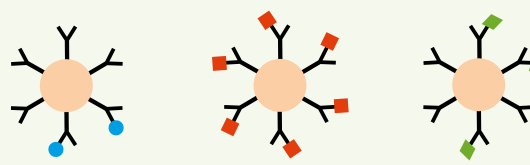
Method

In this MLR assay, T cell-depleted splenocytes (BALB/c mice) and CD3⁺ T cells (C57BL/6 mice) were seeded in a 1:2 ratio respectively. Subsequently, anti-mouse CD3 (clone 145-2C11) was added to the cell mixture together with compounds 1, 2, and 3.

MACSPlex Cytokine Capture Beads coupled to anti-cytokine antibodies



Cytokines bind to specific antibodies on MACSPlex Cytokine Capture Beads



Cytokines bound to specific MACSPlex Cytokine Capture Beads are labeled with MACSPlex Cytokine Detection Reagent

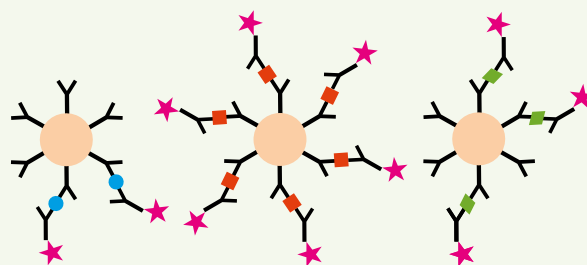


Figure 2: Principle of MACSPlex Assays.

Supernatant was harvested after eight days' incubation and analyzed with the MACSPlex Cytokine 10 Kit, mouse (#130-101-740) according to the [manufacturer's instructions](#) (fig. 2). In a single experiment, we were able to evaluate 10 different cytokines: GM-CSF, IFN- γ , IL-2, IL-4, IL-5, IL-10, IL-12, IL-17A, IL-23, and TNF- α . Samples were acquired by MACSQuant[®] X Flow Cytometer and analyzed using an Express Mode integrated into the MACSQuantify[™] Software.

Results

All three tested compounds showed different cytokine responses in the MLR assay. Compound 3 demonstrated a clear response for GM-CSF, IFN- γ , and IL-17A production (fig. 3). However, a hook effect was observed upon treatment with higher doses for this compound.

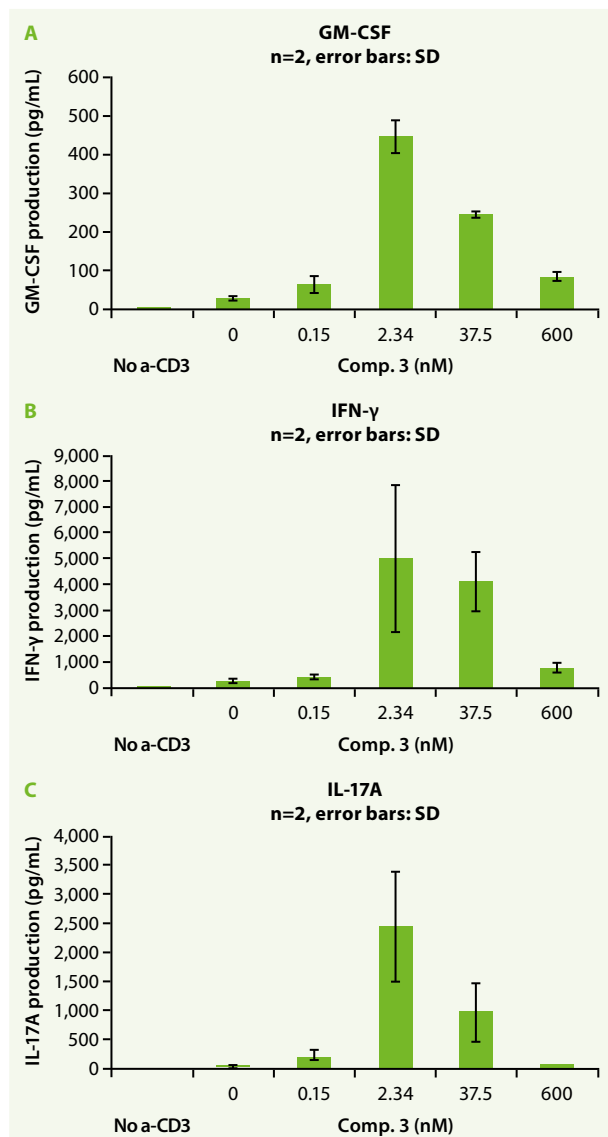


Figure 3: Effect of compound 3 on the production of GM-CSF, IFN- γ , and IL-17A in the mouse MLR assay.

On the other hand, compound 2 showed a clear dose-dependent response for the production of both GM-CSF and IFN- γ (fig. 4A, 4B). A combination treatment of compound 1 and 2 has shown a higher IFN- γ production compared to single treatment with compound 1, which is not observed for GM-CSF production (fig. 4C, 4D).

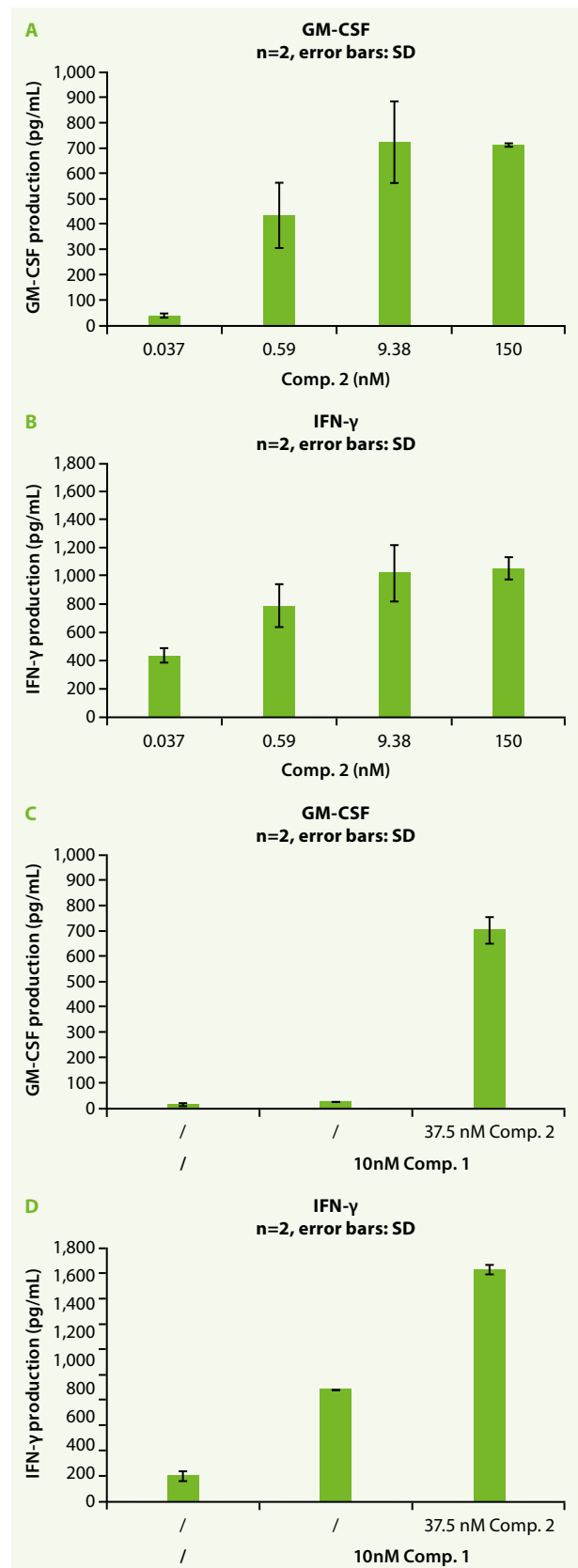


Figure 4: Effect of compounds 1 and 2 separately, and the combination of compounds 1 and 2, on the production of GM-CSF and IFN- γ in the mouse MLR assay.

The obtained results were also confirmed by ELISA (data not shown). No dose-dependent production of IL-2, IL-10, IL-4, IL-5, IL-12, IL-23, and TNF- α was detected after treatment with these compounds.

Conclusions

This study demonstrated that, using the MACSPlex Cytokine Kit and MACSQuant® X Flow Cytometer, multiple cytokine responses from MLR assays can be measured efficiently and accurately in a single experiment and at high-throughput.

MACS Product	Order no.
MACSPlex cytokine 10 kit, mouse	130-101-740
MACSQuant X	130-105-100

Table 1: Related products



Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | www.miltenyibiotec.com

Miltenyi Biotec provides products and services worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use. MACS, MACSQuant, MACSQuantify and the Miltenyi Biotec logo are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this document are the property of their respective owners and are used for identification purposes only. Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.