Bio-Plex™ suspension array system tech note 5374

Use of the Bio-Plex Cytokine Immunoassay to Determine Cytokine Expression Levels in the Intestinal Mucosa

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Introduction

The intestinal tract is a major interface between the finely regulated internal milieu of the body and the external environment. As such, the intestine has a highly developed and specialized mucosal defense system, composed of the epithelial barrier and the intestinal immune system. The intestine faces an enormous, constant antigenic load, both from bacteria and food antigens; however, only a small minority of antigens in the gut represent a threat requiring immune activation and inflammation. Thus, the intestinal immune system is finely regulated to respond appropriately to either harmful or benign antigens (Sansonetti 2004).

Cytokine signaling between the various immune cells is an important facet of immune system regulation; thus, determining the cytokine profile of the intestinal mucosa is important in understanding intestinal immune responses. Multiplex cytokine analysis offers the ability to assay a broad array of cytokines in a single sample; however, this technology has not previously been applied to the study of cytokine expression within the mucosa of the intestine. The goal of this study was to explore the use of the Bio-Plex cytokine assay system for the determination of cytokine levels in the intestinal mucosa. We examined cytokine expression in control mice and in mice treated with anti-CD3, which elicits systemic T-cell activation (Ferran et al. 1990) and diarrhea (Clayburgh et al. 2005), and found that the Bio-Plex system is easily adapted to assess cytokine profiles in the intestinal mucosa.

Methods

Animals

Eight-week-old C57BL/6 mice were injected intraperitoneally with 200 µg of anti-CD3 or phosphate-buffered saline (PBS). Three hours after injection, mice were euthanized for organ harvest. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago.

Tissue Harvest and Cell Lysis

Each mouse was euthanized and ~1.5 cm sections of ileum were removed. The ileum was then cut into ~3 mm^3 pieces and placed in a 1.5 ml Eppendorf tube containing 500 µl of lysing solution (Bio-Plex cell lysis kit). Samples were ground using a microtube pestle and then frozen at –80°C. After thawing, samples were sonicated on ice using 3 short pulses (<3 sec) from a Sonifier unit model 250 (Branson Ultrasonics Corporation). Samples were then centrifuged at 4,500 g for 6 min at 4°C. The supernatant was transferred to a fresh tube, and the protein concentration was determined using a *DC*™ protein assay kit. The protein concentration of each sample was adjusted to 500 µg/ml with lysing solution, aliquoted, and stored at –20°C.

Multiplex Analysis

A Bio-Plex assay for 23 mouse cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1 (MCAF), MIP-1α, MIP-1β, RANTES, TNF-α) was run according to the recommended procedure. Briefly, a standard curve was created via dilution of premixed standards to 50,000 pg/ml, followed by serial dilution to 8 concentrations ranging from 32,000 to 1.95 pg/ml. The assay was performed in the 96-well filtration plate supplied with the Bio-Plex kit. Premixed beads coated with target antibodies (50 µl) were added to each well, and then washed twice with Bio-Plex wash buffer. Premixed standards or undiluted samples (50 µl) were then added to the wells, followed by shaking at 1,100 rpm for 30 sec and incubation for 30 min with shaking at 300 rpm at room temperature. Wells were then washed 3 times with Bio-Plex wash buffer, and 25 µl of the premixed detection antibodies was added to the wells. This was followed by shaking at 1,100 rpm for 30 sec and incubation for 30 min with shaking at 300 rpm at room temperature. Wells were again washed 3 times with Bio-Plex wash buffer, and 50 µl of streptavidin-PE was added to the wells. This was incubated for 10 min with shaking at 300 rpm. Wells were washed 3 times with Bio-Plex wash buffer, and the beads were resuspended in 125 µl Bio-Plex assay buffer. The samples were then read using the Bio-Plex suspension array system, and the data were analyzed using Bio-Plex Manager™ software with 5PL curve fitting.

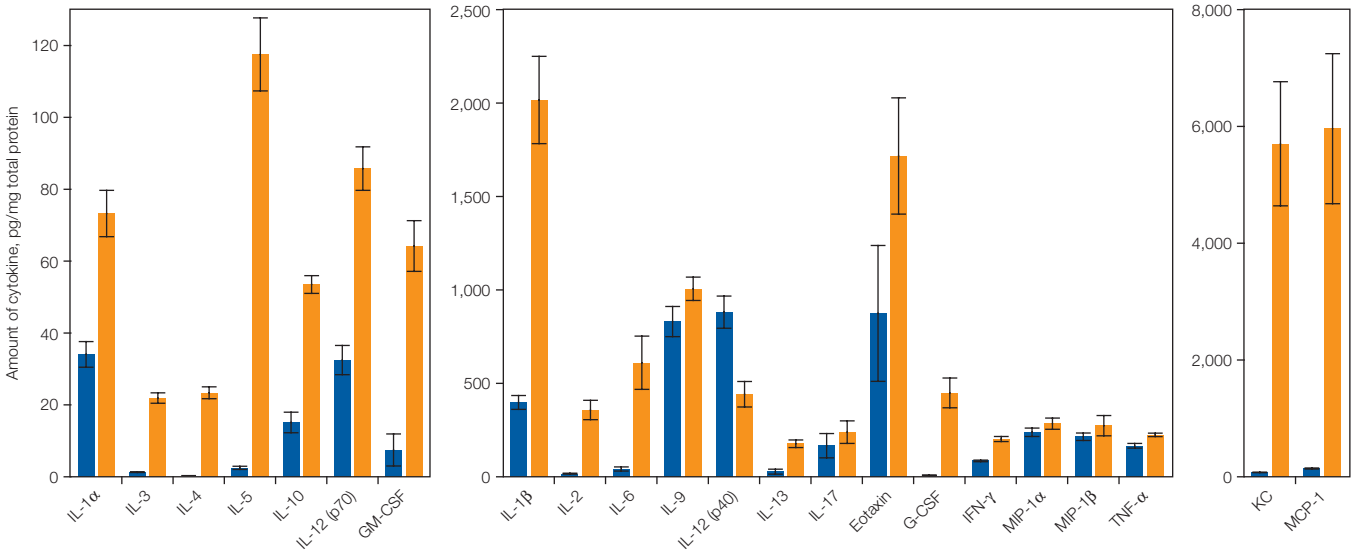

Fig. 1. Cytokine expression in ileal mucosa from control (■) and anti-CD3-treated mice (■). RANTES values were below the level of reliable detection.

Results and Discussion

The results of the Bio-Plex analysis of ileal mucosa from control and anti-CD3-treated animals are shown in Figure 1. The Bio-Plex system effectively detected cytokines across a broad range of concentrations, from ~1.5 pg/mg total protein to over 15,000 pg/mg total protein. As expected, anti-CD3 treatment resulted in significantly increased expression of most cytokines tested, including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-13, IL-17, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, and TNF-α, based on Student's *t*-test.

Conclusions

In this study we have demonstrated the successful determination of expression levels of multiple cytokines within the intestinal mucosa using the Bio-Plex suspension array system. In agreement with previous reports, we found greatly increased expression of numerous cytokines within the mucosa in mice injected with anti-CD3. This procedure should prove important for the analysis of changes within the intestinal immune system during disease or treatment protocols.

References

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Information in this tech note was current as of the date of writing (2005) and not necessarily the date this version was published (rev A, 2006).

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