Bio-Plex Cytokine Immunoassays and ELISA: Comparison of Two Methodologies in Testing Samples From Asthmatic and Healthy Children

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Introduction
Cytokines play a significant role in hematopoiesis, inflammation, and wound healing. These important immunoregulators mediate interactions between various cells, and their dysregulated production may contribute to disease pathogenesis. Elevated levels of cytokines may indicate inflammation or disease progression. Although cytokine functions are complex, cytokine profiles are highly relevant parameters of an immune response. Different cytokines possess biologically overlapping functions, and they have the ability to regulate production of other cytokines. Therefore, analysis of the function of the complete set of cytokines expressed within microenvironments (e.g., a site of inflammation) is often of more value than analysis of a single isolated cytokine (O’Garra and Murphy 1994).

A number of methodologies can be used to measure cytokines. The most common one is the enzyme-linked immunosorbent assay (ELISA), which allows measurement of different cytokines with high sensitivity and specificity. This technology, however, is restricted to analyzing one cytokine target at a time, which limits its usefulness when simultaneous measurement of multiple cytokine targets is desired. The new suspension bead array technology employed in the Bio-Plex system (Luminex xMAP technology) allows multiple cytokine targets to be measured from a single sample in a microplate well (Vignali 2000).

The goal of our study was to compare the Bio-Plex cytokine assays with cytokine ELISA developed in-house for testing cell culture samples from asthmatic and healthy children.

Methods

Cell Isolation and Cultures
Human peripheral blood mononuclear cells (PBMC) were isolated from fresh blood donated by atopic asthmatic children and non-atopic healthy children between the ages of 13 and 15 (Böttcher et al. in press). The cells (1 x 10⁶/ml) were cultured in AIM-V serum-free medium (Life Technologies AB, Sweden) with 20 mM mercaptoethanol (Sigma-Aldrich, Stockholm, Sweden). The cells were stimulated with timothy grass, cat, and dog allergen extracts from ALK (Horsholm, Denmark) at 10,000 standardized units/ml each. The cells were cultured at 37°C with 5% CO₂ for 144 hr. The supernatants were collected after centrifugation and stored at −70°C. Samples were tested simultaneously for cytokines IL-5, IL-10, IL-13, and IFN-γ using both a Bio-Plex human cytokine 4-plex assay and an ELISA made from commercially available antibodies.

Multiplex Assay
A Bio-Plex human cytokine assay for simultaneous quantitation of IL-5, IL-10, IL-13, and IFN-γ was run according to the recommended procedure. In brief, the premixed standards were reconstituted in 0.5 ml of culture medium, generating a stock concentration of 50,000 pg/ml for each cytokine. The standard stock was serially diluted in the same culture medium to generate 8 points for the standard curve. The assay was performed in a 96-well filtration plate supplied with the assay kit. Premixed beads (50 µl) coated with target capture antibodies were transferred to each well of the filter plate (5,000 beads per well per cytokine) and washed twice with Bio-Plex wash buffer. Premixed standards or samples (50 µl) were added to each well containing washed beads. The samples were used directly without further dilution. The plate was shaken for 30 sec and then incubated at room temperature for 30 min with low-speed shaking (300 rpm). After incubation and washing, premixed detection antibodies (50 µl, final concentration of 2 µg/ml) were added to each well containing washed beads. The samples were used directly without further dilution. The plate was shaken for 30 sec and then incubated at room temperature for 30 min with low-speed shaking (300 rpm). After incubation and washing, premixed detection antibodies (50 µl, final concentration of 2 µg/ml) were added to each well. The incubation was terminated after shaking for 10 min at room temperature. After 3 washes, the beads were resuspended in 125 µl of Bio-Plex assay buffer. Beads were read on the Bio-Plex suspension array system, and the data were analyzed using Bio-Plex Manager™ software (v 3.0) with 5PL curve fitting.
**Conclusions**

In this study, we were able to demonstrate that testing PBMC samples from asthmatic and healthy children for IL-5, IL-10, IL-13, and IFN-γ by ELISA and Bio-Plex assays yielded similar results. Bio-Plex assays have broader dynamic range and allow for multiplex analysis of cytokines in a single sample. Therefore, Bio-Plex assays have an advantage over the current ELISA methodology when multiple samples have to be analyzed for several cytokines and when available sample volume is limited.

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**References**

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