**Introduction**

Human milk contains numerous immune factors, which together constitute the "immune system of milk" [1].

- White blood cells
- Antibodies (secretory immunoglobulin A)
- Immune cell communication molecules (cytokines)
- Antimicrobial factors (e.g., lactoferrin, fibronectin)
- Commensal microbes

The immune system of milk protects infants against infectious disease and guides their immune system development. Immune cells in milk can enter lymph tissue (Peyer’s patches) in the infant gut to coordinate immune responses [2]. Maternal immune cells "train" infant immune cells, transferring lasting memory [3].

We have developed a technique to describe immune responses in whole human milk that is:

- Interpretable at the system level (the immune system of milk)
- Practical for population-based, international research

In this technique, milk specimens are combined with pathogenic or commensal gut bacteria, incubated at 37°C for 24 hours, and evaluated for cytokines. Comparison of cytokine concentrations in stimulated and baseline specimens provides a measure of immune cell activity—the immune response in milk.

**Methods**

Participating women provided milk specimens by expression with an electric breast pump and provided information about their child, health, and breastfeeding practices.

One milliliter of milk was isolated by centrifugation and the aqueous portion was frozen as a "Baseline" specimen. Within four hours of expression, two milliliters of milk were diluted with mammalian cell culture medium (RPMI 1640 was frozen as a "Baseline" specimen. IL-6 ratios were most commonly observed. IL-10 ratios to Baseline (arithmetic mean, range): Salmonella: 1.51 (1, 386.5); E. coli: 1.29 (1, 260.7); Bifidobacterium: 1.35 (1, 275.3); Lactobacillus: 1.31 (1, 306.2).

**Baseline IL-6 was positively correlated with IFN-γ responses to Salmonella (ρ = 0.49; p = 0.0083), but not Salmonella (ρ = 0.07; p = 0.7166).**

Baseline IL-6 was positively correlated with IFN-γ responses to Salmonella (ρ = 0.49; p = 0.0065), Bifidobacterium (ρ = 0.39; p = 0.0323), and Lactobacillus (ρ = 0.40; p = 0.0345), but not E. coli (ρ = 0.10; p = 0.6053). Baseline IL-6 was also positively associated with IL-10 responses.

**Baseline predictors of cytokine responses:**

**Baseline IL-6 was generally positively associated with all three cytokine responses (Figure 3).**

Baseline IL-6 was positively correlated with IFN-γ response to E. coli (ρ = 0.39; p = 0.0323), and IFN-γ response to Lactobacillus (ρ = 0.49; p = 0.0083), but not Salmonella (ρ = 0.07; p = 0.7166).

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**Baseline IL-6 ratios to Baseline (geometric mean, range):**

Basal saline responses were not as strongly correlated with IL-6 responses to E. coli, Bifidobacterium, or Lactobacillus as responses to these stimuli were correlated with each other.

**Interleukin-6, IL-10, and IFN-γ responses (ratios to Baseline) were greater in the Salmonella condition.** This was significant for IL-6 (all p < 0.05).

**Conclusions:**

The immune system of milk generally mounts pro-inflammatory responses in vitro. Increases in the pro-inflammatory cytokine IL-6 were more common that increases in the anti-inflammatory cytokine IL-10 and the type 1 cytokine IFN-γ.

**IL-6 responses to Salmonella stood out in potentially important ways:** They were larger in magnitude (Figure 1) than responses to other bacteria; they were generally positively associated with IL-6 responses to other bacteria (Table 1); and, they were unassociated with other cytokines responses to Salmonella (Figure 2).

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References:


