Biological activities of a novel bovine colostrum-based proprietary concentrate, ARMRA, at the cellular level

Gitte S. Jensen, PhD, Research director, Department of Natural Products Research, NIS Labs.

Synopsis

A novel nutraceutical blend of bioactive fractions from bovine colostrum was designed for immune protection, gut integrity, and cellular health. The product, ARMRA, contains oligosaccharides and peptides from the low-molecular weight fraction of bovine colostrum with gut-protective colostral immunoglobulins.

A research project was recently completed at NIS Labs where the proprietary concentrate showed a promising combination of protective effects at the cellular level, involving both immune cells and gut cells, and suggesting a potential for multi-system cellular communication and cross-talk between these cell types.

Introduction

As we continue to face the global challenges of infectious diseases, and the daily threats of new environmental toxicants, protection from and the recovery after such assaults have taken on an importance of unprecedented proportions. The gut, skin, and airways represent protective body surfaces engineered to help protect against penetration by pathogens and environmental threats. The immune system represents the body's interior system of defense and assists the protective effects of these barriers, as well as neutralizes those harmful particles that breach these barriers.

In Nature, the transfer of immune protection from immune-competent mothers to their young is necessary to protect vulnerable newborn mammals after birth. This is accomplished through feeding on colostrum, which is the first milk the mother produces after giving birth. Colostrum is not species-specific and homology between bovine and human colostrum is well-documented. Thus, it serves both as a food and a nutritional adjuvant lending a natural therapeutic potential for immune support.[1]

Bovine colostrum from healthy cows has been shown to transfer immune protection to other mammals against potential pathogens that the cow has encountered. Bovine colostrum contains multiple immune-active components, including immunoglobulins, prebiotic oligosaccharides, and bioactive peptides designed for immune protection against bacterial and viral challenges.[2] [3]

A novel bioactive extract from bovine colostrum, ARMRA, was designed to provide a concentrated nutraceutical extract. This paper describes results from testing involving gut cells and immune cells aimed at documenting the properties of ARMRA at the cellular level.

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[1] Bagwe S, Tharappel LJ, Kaur G, Buttar HS. Bovine colostrum: an emerging nutraceutical. J Complement Integr Med. 2015;12(3):175-85.

[2] Benson KF, Carter SG, Patterson KM, Patel D, Jensen GS. A novel extract from bovine colostrum whey supports anti-bacterial and anti-viral innate immune functions in vitro and in vivo: I. Enhanced immune activity in vitro translates to improved microbial clearance in animal infection models. Prev Med. 2012 May;54 Suppl:S116-23.

[3] Jensen GS, Patel D, Benson KF. A novel extract from bovine colostrum whey supports innate immune functions. II. Rapid changes in cellular immune function in humans. Prev Med. 2012 May;54 Suppl:S124-9.

Results

Rapid onset of immune defense activity

Human immune cells were tested for anti-bacterial activity in a lab test where the mononuclear and polymorphonuclear phagocytes were pre-treated with ARMRA for 20 minutes and then challenged with a bacterial peptide to trigger an immune defense reaction from the immune cells.

The bacterial trigger was the tri-peptide combination of the 3 amino acids Methionine, Leucine, and Phenylalanine, f-Met-Leu-Phe (fMLP), which is a standard model for triggering phagocyte formation of intracellular Reactive Oxygen Species (ROS) as a defensive response to a bacterial invader. It functions as a potent polymorphonuclear leukocyte chemotactic factor and is also a macrophage activator.

Method: The phagocytes were pre-treated with ARMRA, loaded with a precursor dye that turns fluorescent when exposed to damaging free radicals, and then fMLP was added to trigger ROS formation. The intracellular fluorescence was measured by flow cytometry.

The results showed that ARMRA treatment of the immune cells alerted the cells to a stronger ROS formation against the inflammatory bacterial peptide. This was seen for both mononuclear phagocytes – i.e. macrophages – and polymorphonuclear cells (**Figure 1**).

The effect of ARMRA was compared to a whole fat-free bovine colostrum extract. ARMRA was superior to colostrum in triggering this rapid anti-bacterial immune defense activity. This was most obvious at the lowest dose tested, where colostrum had lost its activity in this assay, but ARMRA still provided over 32% increase in ROS formation in the mononuclear phagocytes.

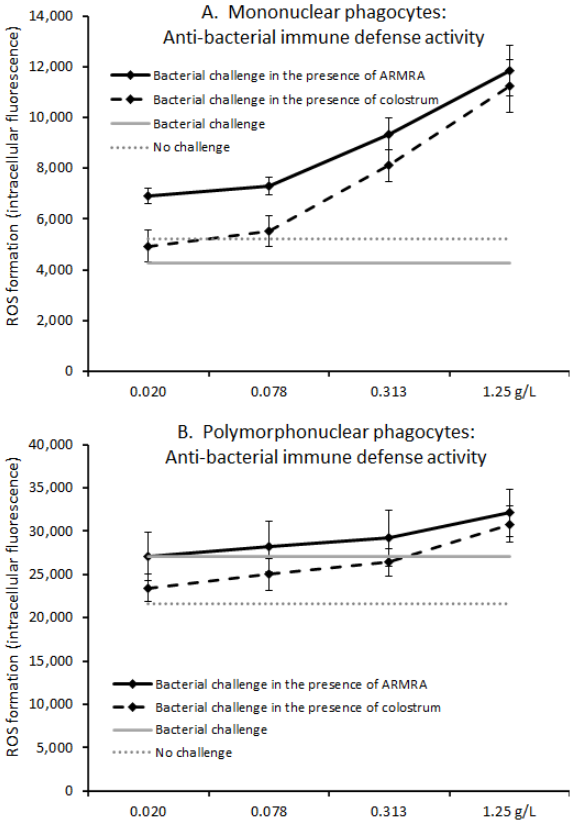

Figure 1. Formation of Reactive Oxygen Species (ROS) by human phagocytic immune cells in response to a bacterial peptide as a model for bacterial infection. A: Mononuclear cells, and B: Polymorphonuclear cells. Both cell types responded to ARMRA-treatment by increased ROS formation. Untreated cells ("No challenge", dotted line) served as a baseline control. Cells exposed to the trigger of a bacterial defense mechanism in the absence of ARMRA served as a reference ("Bacterial challenge", grey line).

Protection of cellular energy production in immune cells and gut cells

When cells experience inflammation, many cellular functions fall under increased metabolic demand. Oftentimes, cellular energy production is insufficient to cope with the stress, impairing cellular health and performance.

Two types of cells were tested: 1) Immune cells isolated from white blood cells from a healthy blood donor, and 2) Gut epithelial cells (the T84 cell line). For the gut epithelial cells, inflammatory stress was induced with the bacterial toxin LPS (lipopolysaccharide).

ARMRA-treated cells showed 11% higher cellular viability and metabolic activity in healthy immune cells (**Figure 2, left**), and 22% higher cell viability in gut epithelial cells under inflamed conditions (**Figure 2, right**).

This test was performed using the MTT bioassay where the colorimetric measurement is proportional to cellular metabolism and energy production, evidencing that ARMRA confers cellular resiliency, protecting fundamental aspects of cellular health, also under inflammatory stress conditions.

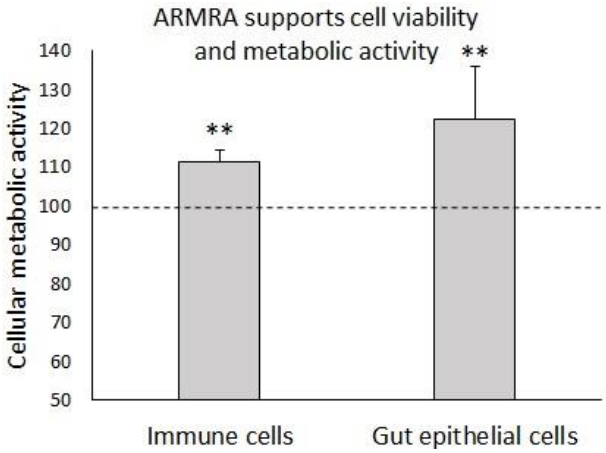

*Figure 2. Cell viability as a relative measure of cellular metabolism and energy production. Cell cultures not treated with ARMRA served as a negative control (dashed line) are set to 100% cellular metabolic activity. The increase in cellular energy production in the ARMRA-treated cell cultures when compared to untreated control cultures was highly significant as indicated by ** (P<0.01).*

Gut epithelial cell migration during recovery from a mechanical wound in the presence of an inflammatory bacterial toxin

The protective effects of ARMRA on gut epithelium was tested in the lab. The human T84 cell line was used as a model,[4] and the cells were allowed to differentiate in culture dishes until a complete monolayer of gut epithelial cells had formed. The cells then differentiated to a mature state where they formed microvilli and tight junctions for a skin-like 2-dimensional model of the gut barrier.

Mechanical wounding was used as a model as well as inflamed conditions where the bacterial toxin LPS was used to induce inflammation. Cell cultures were treated with ARMRA immediately before and after the mechanical wound was created. Cultures not treated with ARMRA served as controls.

The gut epithelial cells were allowed to repair the wound over the following 6 days in culture. The inflamed cultures that were not treated with ARMRA before and after the wound was created showed a very slow healing process, with the wound only 10% healed after 6 days. In contrast, the cell cultures that received ARMRA before and after wound formation showed 60-70% repair after 6 days (**Figure 3**).

[4] Devriese S, Van den Bossche L, Van Welden S, Holvoet T, Pinheiro I, Hindryckx P, De Vos M, Laukens D. T84 monolayers are superior to Caco-2 as a model system of colonocytes. Histochem Cell Biol. 2017 Jul;148(1):85-93.

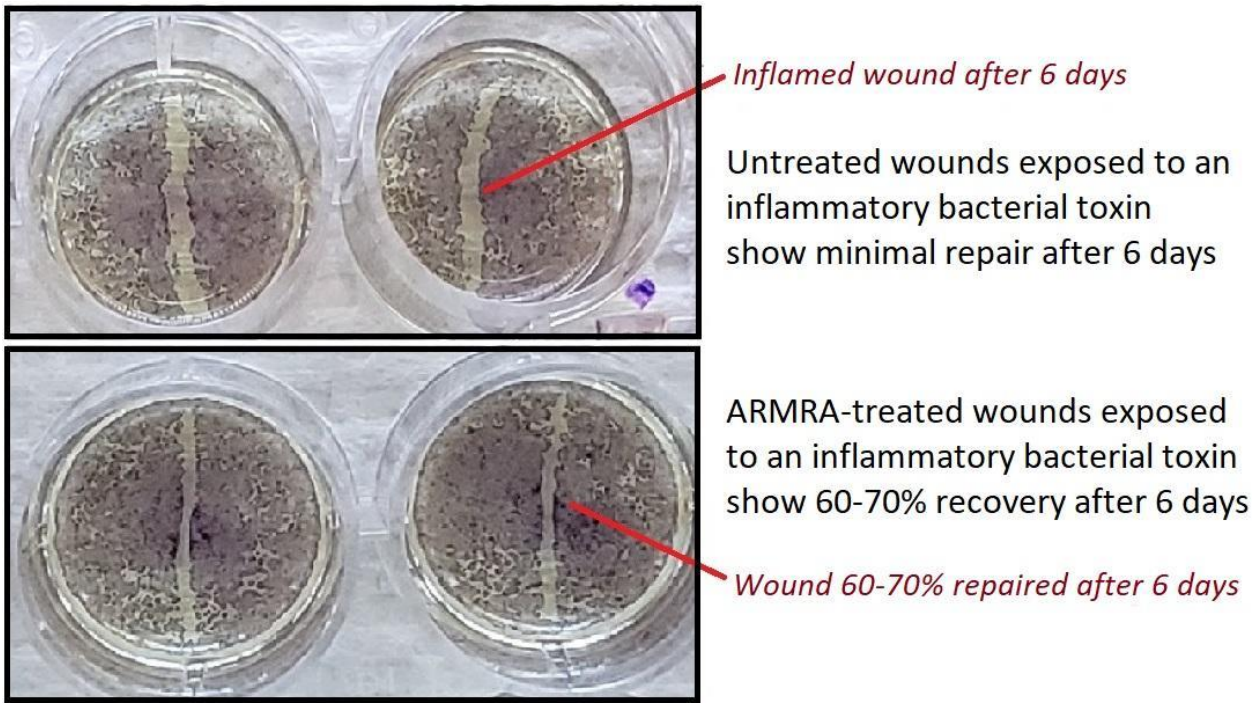

Figure 3. Gut epithelium matured to a complete layer in culture dishes and subsequently wounded by a mechanical scratch. The wound healing process was accelerated when the cell cultured were treated with ARMRA immediately before and after the wound was made.

Effects on gut epithelial cell production of selected cytokines and anti-viral peptides

The gut barrier has molecular mechanisms of communicating with the immune system and alert immune cells of the need to migrate to areas in need of defense and repair. The ability of ARMRA to support this level of cellular communication was evaluated. Gut epithelial cells can secrete multiple kinds of signaling molecules, including cytokines, to communicate with the rest of the body. T84 gut epithelial cells were cultured, some cultures were treated with ARMRA, and upon inflammatory stress the secreted cytokines were quantified.

ARMRA treatment of human gut epithelial cells triggered rapid secretion of cytokines and anti-viral peptides, important for orchestrating communication between gut epithelial cells and the immune system.

Within only **2 hours of** treatment with ARMRA, the T84 cell cultures showed detectable levels of many secretory communication molecules. Just **24 hours** after ARMRA treatment the results showed a highly selective pattern of cytokines (**Figure 4**).

Under both normal and inflamed culture conditions, ARMRA-treated cells showed increased production of:

- **Interleukin-8 (IL-8),** involved in instant cellular communication. IL-8 exists pre-made inside epithelial cells, ready to be secreted as a chemokine (chemoattractant), to help recruit immune cells into the area. IL-8 also helps stimulate phagocytosis once the immune cells arrive to the site. ARMRA-treated T84 cells secreted over

60% more IL-8 under inflamed conditions than LPS control cultures.

- **Interferon gamma-induced protein-10 (IP-10),** a chemokine that attracts macrophages, T cells, Natural Killer cells, and dendritic cells to the area. ARMRA-treated T84 cells secreted over 240% more IP-10 under inflamed conditions than LPS control cultures.
- **Macrophage Inflammatory Protein-alpha (MIP-1α)**, a chemokine for recruiting macrophages and with vital roles in immune responses towards infection and inflammation. ARMRA-treated T84 cells secreted over 220% more MIP-1α under inflamed conditions than the LPS control cultures.

In contrast, typical pan-inflammatory markers, including the cytokines IL-1β and TNF-a associated with widespread and non-discriminatory inflammation, were not detectable after ARMRA treatment of the gut epithelial cells. This supports the observation of a selective immune-alerting communication by ARMRA.

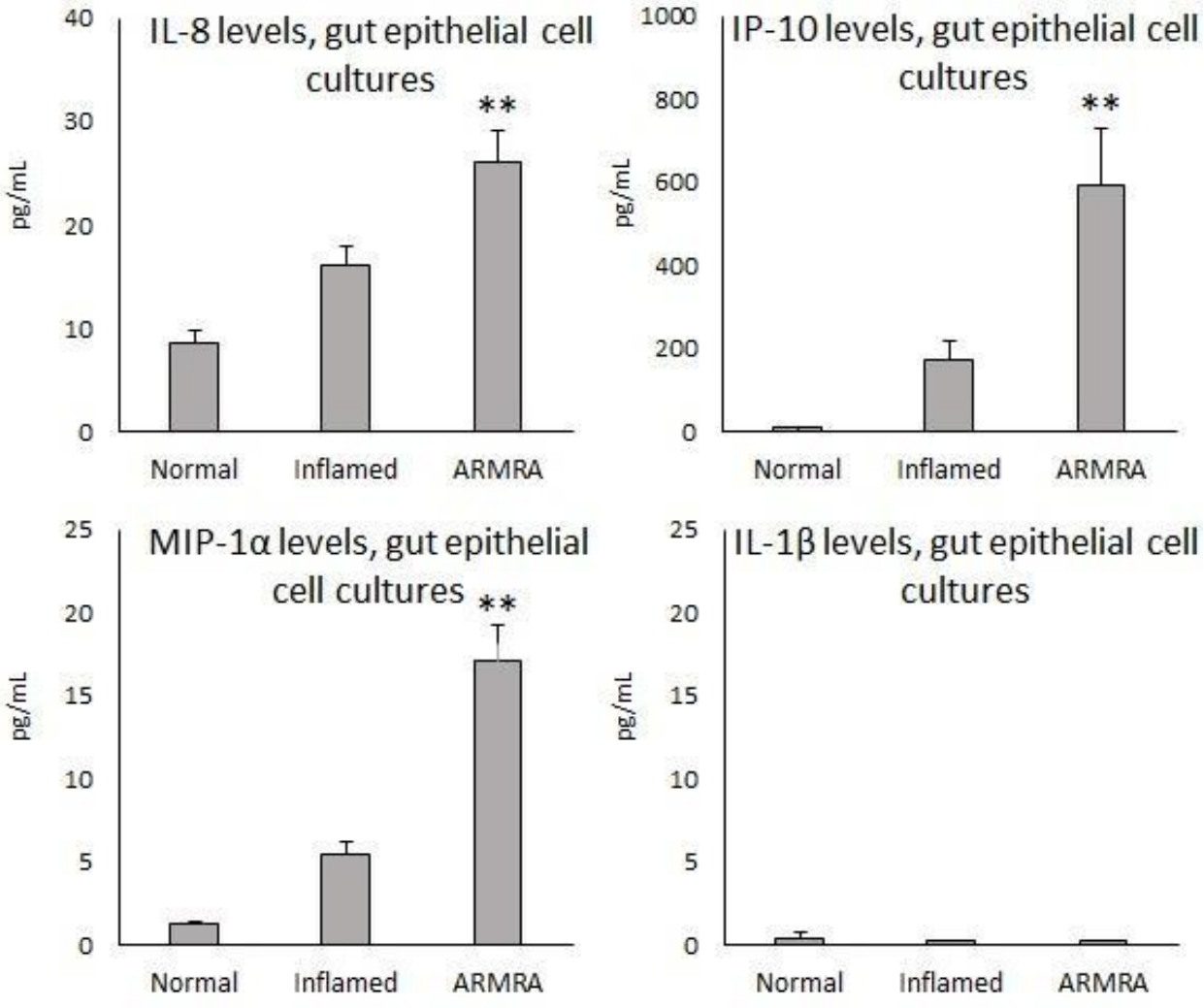

Figure 4. ARMRA-treatment of inflamed gut epithelial cells led to induction of the chemotactic factors IL-8, IP-10, and MIP-1α. In contrast, inflammatory cytokines such as IL-1β (shown) and TNF-α (not shown) were undetectable.

Discussion

The integrity of the mucosal barriers and the interconnected gut and immune related supportive communication pathways serve to protect us from infections, environmental toxicants. and the resulting damage from unregulated inflammation.

The ARMRA extract of colostral bioactives triggered rapid responses in conferring protection via to both immune cells and gut epithelial cells. The changes were measurable within just 2 hours of treatment with ARMRA in cell cultures.

For immune cells, the increased ROS formation in response to a bacterial peptide translates into elevated immune defense activity. ARMRA was superior to whole fat-free bovine colostrum in this cellular model of immune alertness.

For gut epithelial cells the rapid and highly selective increase in secretory chemokines demonstrates cross-talk between the epithelium and the immune system.

ARMRA showed support of the intestinal barrier function in four synergistic ways:

1. ARMRA supported immune cell alertness and anti-bacterial attack functions.
2. ARMRA supported cellular viability, metabolism, and energy production to cells under inflammatory stress.
3. ARMRA strengthened the integrity of the gut epithelial barrier and assisted accelerated repair after wounding.
4. ARMRA supported gut epithelium communicating with the immune system.