CORRELATION BETWEEN POLYMORPHONUCLEAR LEUKOCYTES VIABILITY WITH SOMATIC CELL COUNT ON BOVINE MILK

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Somatic milk cells are crucial for an effective defense in mammary gland. The initial increases in milk somatic cell count (SCC) are primarily due to the recruitment of circulating polymorphonuclear leukocytes (PMNL) from the circulation to the inflamed tissue. The PMNL produce ROS and granule components that are highly effective at killing pathogens, however there is concomitant potential to cause severe tissue destruction should they undergo necrotic lysis and release cytotoxic granule contents and ROS onto host tissues. Thus, it follows that timely and vigilant execution of a controlled cell death program in PMNL after phagocytosis is important for preventing damage to healthy tissues, and necessary for resolution of infection. In this context, apoptosis provides a granulocyte clearance mechanism in tissue which would tend to limit the inflammation tissue injury and promote resolution rather than persistence of infection. The purpose of the present trial was to evaluate the correlation between SCC and percentages of necrotic and/or apoptotic PMNL. Thus, 12 milk samples were collected and diluted with phosphate-buffer saline at a proportion 1:1. Firstly, the separation of milk cells was performed after centrifugation steps. Then, the PMNL were identified by flow cytometry using anti-bovine granulocyte monoclonal antibody (CH138A) and APC secondary antibody. Apoptosis and necrosis were quantified using dual-color flow cytometric procedure with fluorescein labeled annexin-V and propidium iodide (PI). Milk samples were also collected for SCC and examined by an automatic cell counter. The correlations were determined by Pearson's correlation. The correlation between SCC and the percentage of viable PMNL (Annexin-V-/PI-) was $r = -0.73$ ($P = 0.007$), between SCC and the percentage of PMNL undergoing apoptosis (Annexin-V+/PI-) was $r = 0.64$ ($P = 0.026$), and between SCC and the percentage of PMNL undergoing necrosis (Annexin-V-/PI+) was $r = -0.36$ ($P = 0.25$), and between SCC and the percentage of PMNL suffering apoptosis or necrosis (Annexin-V-/PI+) was $r = -0.13$ ($P = 0.69$). In face of, this study emphasizes the idea of pre-existing milk PMNL as a part of the innate udder's defense against bacteria, since the active immune defense requires viable and immune competent somatic cells for rapid and efficient elimination of the pathogen.