

December 18, 2024

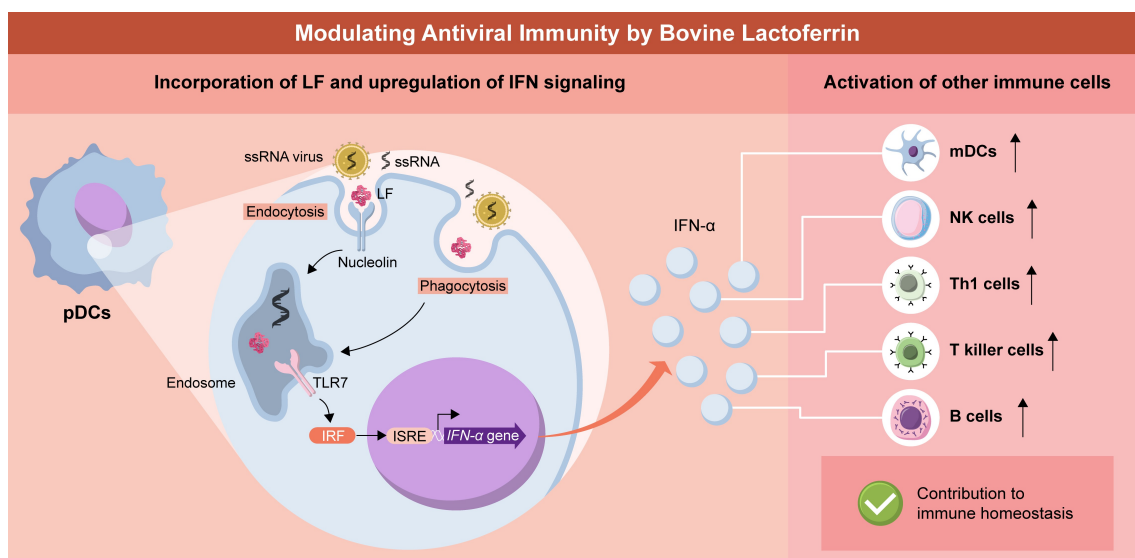
Morinaga Milk, world’s top producer*¹ of lactoferrin, discovers mechanism by which lactoferrin acts on plasmacytoid dendritic cells and enhances immunity

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Morinaga Milk Industry Co., Ltd. has investigated lactoferrin (a type of milk protein) for more than 60 years. We are pleased to report that our joint research with Wakayama Medical University (specifically, Professor Shinichi Hashimoto) has revealed the following points about the mechanism by which lactoferrin enhances immunity. These findings were published in the *International Journal of Molecular Sciences* on December 13, 2024.*²

- Lactoferrin is taken up by plasmacytoid dendritic cells (pDCs, a type of immunocyte) through phagocytosis and the lactoferrin receptor nucleolin.
- Lactoferrin increases the sensitivity of toll-like receptor 7 (TLR7), the virogene sensor present in pDCs, and amplifies pDC activation and interferon- α (IFN- α , an immune substance) production.
- Lactoferrin enhances the activation of a broad range of immunocytes downstream of pDCs that are regulated by IFN- α .

Through this mechanism, lactoferrin—a protein found in exocrine secretions such as tears, nasal mucus, and saliva—may play a role in enhancing immune responses to viruses. Milk-derived lactoferrin taken orally should also help improve and maintain health by performing similar functions in the digestive tract. Accordingly, we will continue our research on the ways lactoferrin contributes to human health.



1. Research background

Lactoferrin is a type of protein found in milk, and is also found in exocrine secretions such as tears, nasal mucus, and saliva. Given that the mucosa these secretions cover are constantly exposed to viruses and other foreign substances, lactoferrin may play a role in modulating immune responses to viruses.

In previous clinical studies, we demonstrated that intake of bovine milk-derived lactoferrin modulates the activity of pDCs in peripheral blood and relieves cold-like symptoms.^{*3}

Many viruses that cause cold-like symptoms have single-stranded RNA genomes. pDCs use intracellular toll-like receptor 7 (TLR7) to recognize virus-derived single-stranded RNA, and produce large amounts of the immune substance IFN- α . IFN- α enhances immune responses to viruses by activating a broad range of immunocytes in the innate and acquired immune systems. Therefore, lactoferrin may relieve cold-like symptoms by causing pDCs to enhance immunity.

In this study, we conducted cell culture experiments to examine how bovine milk-derived lactoferrin affects pDCs and enhances immunity.

2. Research method

We collected peripheral blood from healthy adults and used it to prepare mononuclear cells (a mixture of pDCs and other immunocytes). We cultured these mononuclear cells with R-848, a substance that stimulates TLR7 in the same manner as virus-derived single-stranded RNA, and bovine milk-derived lactoferrin, and evaluated their effects on the pDCs and other immunocytes in the mononuclear cells. We also examined the effects of lactoferrin on IFN signaling using TLR7 reporter cells that, like pDCs, express TLR7 and make it possible to observe IFN signaling from TLR7 stimulation to IFN- α gene induction.

3. Research results

(1) Lactoferrin is taken up by pDCs through phagocytosis and the lactoferrin receptor nucleolin.

When we cultured mononuclear cells with fluorescently labeled lactoferrin under TLR7 stimulation, the fluorescence intensity of the pDCs in the mononuclear cells increased, indicating that they had taken up the lactoferrin (Figure 1). pDCs take in foreign substances from their surroundings by means of phagocytosis; when we inhibited this function, the fluorescence intensity of the pDCs decreased. Additionally, pDCs express a lactoferrin receptor called nucleolin on cell surfaces; when we inhibited this function, the fluorescence intensity of pDCs decreased as well. These results indicate that lactoferrin is taken up by pDCs through phagocytosis and the lactoferrin receptor nucleolin (Figure 1). Notably, several single-stranded RNA viruses are also known to attach to and invade cells through nucleolin; lactoferrin may affect the dynamics of single-stranded RNA within cells.

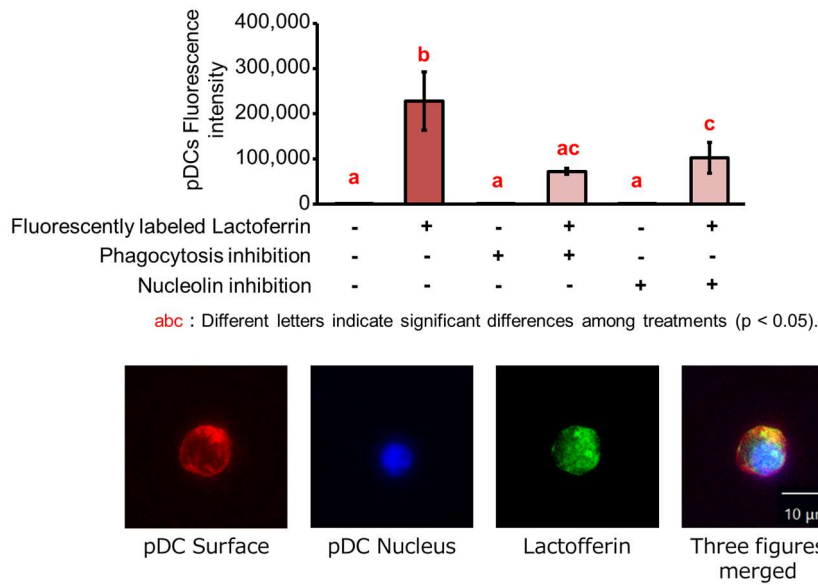
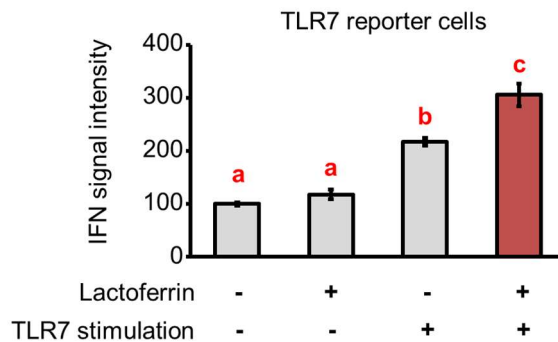


Figure 1. Uptake of lactoferrin by pDCs

(2) Lactoferrin increases TLR7 sensitivity and amplifies IFN signaling

To investigate whether cellular uptake of lactoferrin enhances intracellular IFN signaling, we used TLR7 reporter cells to evaluate the effects of TLR7 stimulation and lactoferrin. In the absence of TLR7 stimulation, adding lactoferrin did not induce IFN signaling. By contrast, with TLR7 stimulation, IFN signaling was induced, and adding lactoferrin amplified IFN signaling (Figure 2). Similarly, in experiments with mononuclear cells, adding lactoferrin under TLR7 stimulation enhanced pDC activation and IFN- α production, and also increased the concentration of IFN- α in the culture supernatant (Figure 3). These findings suggest that lactoferrin amplifies IFN signaling in pDCs by increasing the sensitivity of TLR7, which recognizes virus-derived single-stranded RNA.



abc : Different letters indicate significant differences among treatments (p < 0.05). The vertical axis indicates the IFN signal intensity with the additive-free condition as 100%.

Figure 2. Amplification of IFN signaling by lactoferrin

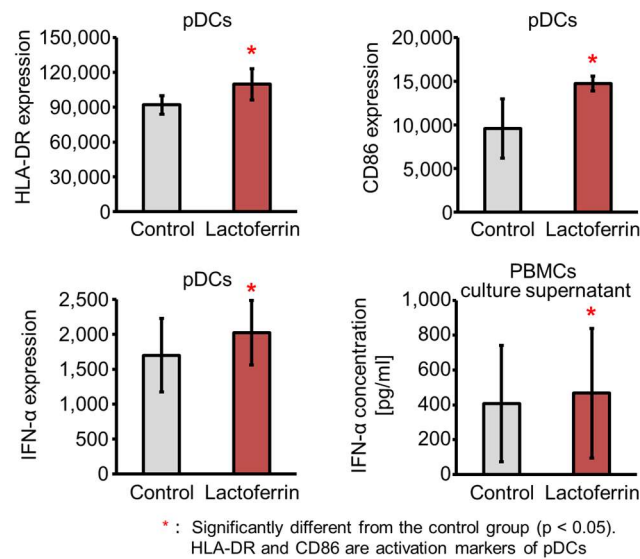


Figure 3. Enhancement of pDC activation and IFN- α production caused by lactoferrin

(3) Lactoferrin increases the activation of a broad range of immunocytes downstream of pDCs

IFN- α is known to activate a broad range of immunocytes in the innate and acquired immune systems. Given that lactoferrin enhances pDC activation and IFN- α production, we expected that it would also enhance the activation of other immunocytes in mononuclear cells. To test this, we added lactoferrin when culturing mononuclear cells under TLR7 stimulation, and found that doing so enhanced the activation of myeloid dendritic cells (mDCs), natural killer (NK) cells, T killer cells, T helper 1 (Th1) cells, and B cells (Figure 4). These findings indicate that when TLR7—which recognizes virus-derived single-stranded RNA—is stimulated, lactoferrin enhances the activation of pDCs as well as a broad range of downstream immunocytes.

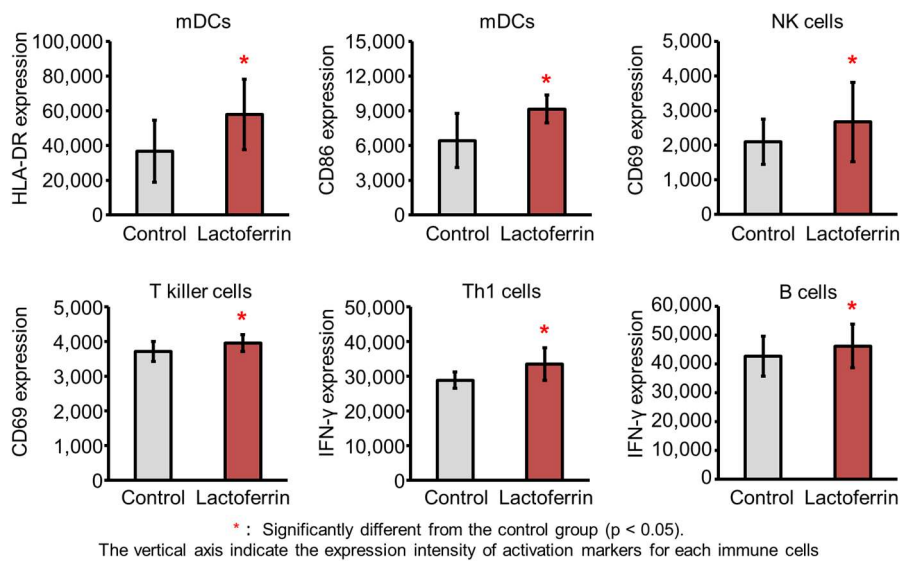


Figure 4. Enhanced activation of a broad range of immunocytes by lactoferrin

These results suggest that lactoferrin enhances immunity through a mechanism by which it is taken up by pDCs through phagocytosis and the lactoferrin receptor nucleolin, and increases the sensitivity of TLR7—which recognizes virus-derived single-stranded RNA—thereby enhancing pDC activation and IFN- α production and activating a broad range of immunocytes in the innate and acquired immune systems, including downstream mDCs, NK cells, killer T cells, Th1 cells, and B cells.

Through this mechanism, lactoferrin—a protein found in exocrine secretions such as tears, nasal mucus, and saliva—may play a role in enhancing immune responses to viruses. Milk-derived lactoferrin taken orally should also help improve and maintain health by performing similar functions in the digestive tract. Accordingly, we will continue our research on the ways lactoferrin contributes to human health.

Morinaga Milk Industry's lactoferrin initiatives

Morinaga Milk has studied, manufactured, and applied lactoferrin to foods for more than 60 years. Companies around the world are now using lactoferrin as a functional food ingredient. Researchers around the world are vigorously studying various aspects of lactoferrin, and we will collaborate with them to further promote lactoferrin research and contribute to human health. Notably, we have published more research papers on lactoferrin than any other company in the world.*4

Reference

***1 Absolute Reports. (2023) Share of production volume of Morinaga Milk Industry Co., Ltd. subsidiary MILEI GmbH**

***2 Takumi Yago, Asuka Tada, Shutaro Kubo, Hirotsugu Oda, Sadahiro Iwabuchi, Miyuki Tanaka, and Shinichi Hashimoto. “Bovine lactoferrin enhances Toll-like receptor 7 response in plasmacytoid dendritic cells and modulates cellular immunity.” *Int J Mol Sci.* 2024; 25(24), 13369. URL: <https://doi.org/10.3390/ijms252413369>**

***3 *Nutrients.* 2023; 15(18):3959. URL: <https://doi.org/10.3390/nu15183959>**

***4 Search for “lactoferrin” in SCOPUS database (Elsevier) (as of December 2024)**

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