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Protection against infections by oral lactoferrin: Evaluation in animal models

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Abstract

It has been reported previously that oral administration of lactoferrin (LF) provides some host-protective effects against infections, cancers, and inflammations. In this review, we focus on the effect of oral LF on various infectious diseases and discuss the mechanism as elucidated in animal models. In the case of infections occurring at sites other than the digestive canal, it is unclear whether oral LF is absorbed from the intestine and exerts its protective effect at the site of infection. In preterm human infants, neonatal pigs, and rats with colitis, it was reported that LF is detectable in various body fluids after oral administration. We could not detect the transport of oral bovine LF into the blood of adult rats without gastrointestinal illness using several techniques, suggesting that there is an extremely low level of transport of LF, if any. Orally administered LF may act at the oro-gastro-intestinal mucosa and aid the defense system against infections through a network of mucosal immunity and systemic immunity. Indeed, it is reported that oral LF increases the number of cells in the leukocyte subset and cytokine (IFN-γ and IL-18) production in the intestinal mucosa of mice. Regarding systemic immunity, we have observed an increase of leukocyte number, cytokine (IFN-γ, TNF-α, IL-12, and IL-18) production, and effector activity of macrophages in response to LF administration in several animal models. These enhanced immune responses may contribute to eradication of the pathogen, resolution of the symptoms, and maintenance of the homeostasis during infectious diseases.

Introduction

Oral administration of lactoferrin (LF) has been reported to exert host protective effects against various diseases in animals and human (Tomita *et al.* 2002). These diseases include infections, cancers, and inflammations. This review focuses on the effect against infections in animal models. Up to now, protective effects of oral LF on infectious diseases have been reported in the following animal models and human diseases: fungal infections, including dermatophytosis (Wakabayashi *et al.* 2000, Yamauchi *et al.* 2000) and candidiasis (Takakura *et al.* 2003); viral infections, including chronic hepatitis C (Tanaka *et al.* 1999); and bacterial infections, including infections by *Escherichia coli* (Teraguchi *et al.* 1995), *Staphylococcus aureus* (Bhimani *et al.* 1999), and *Helicobacter pylori* (Wada *et al.* 1999).

Absorption of ingested LF

When we think about the mechanism of the host protective effect of oral LF, two simple questions arise. Is orally administered LF absorbed from the intestine and does it exert its protective effect at the site of infection, especially in the case of diseases occurring at sites distant from the digestive canal?

Actually, the absorption of oral LF has been studied in newborn infants and pigs, and in animal models of colitis. Substantial amounts of nearly intact molecules of maternal human LF were detected in the

Table 1. Upregulation of immunological parameters by oral bovine LF

Region	Animal model or human	Parameters	Reference
Intestinal mucosa	Normal mice	IgA and IgG in Peyer's patch	Debbabi 1998
	Normal or tumor-bearing mice	IL-18, IFN-γ	Wang 2000
		$CD4^+$, $CD8^+$, NK, IgM^+, IgA^+ cells	Kuhara 2000
Peripheral blood	Healthy human or post-surgical patients	Immature granulocytes (bands)	Zimecki 1998, 2001
	Normal or tumor-bearing mice	Leukocyte cytotoxicity	Iigo 1999
		$CD4^+$, $CD8^+$, NK cells	Kuhara 2000
	Chronic hepatitis C patients	IL-18	Ishii 2003
Spleen	Azoxymethane-injected rats	NK cell activity	Sekine 1997
	Normal mice	IFN-γ, IL-10	Nakajima 1999
	Immunized guinea pigs	Proliferation and effector activity	Wakabayashi 2002
Peritoneal cavity	Inactivated *Candida*-injected mice	NO production and anti-*Candida* activity of macrophages	Wakabayashi 2003
Systemic	Normal or cyclophosphamide-injected mice	DTH reaction	Zimecki 2000 Artym 2003

urine of human preterm infants (Hutchens *et al.* 1991a, 1991b). In a study of neonatal pigs, oral bovine LF was shown to be transferred to the bile and cerebrospinal fluid via the blood (Harada *et al.* 2002). In experimental colitis rats, oral bovine LF was detected in the blood at relatively low concentrations (Togawa *et al.* 2002). These observations may be explained as follows. The barrier function of the intestinal tract is not fully developed in newborn animals, and in adult animals, the intestinal barrier function may be damaged by gut injuries such as those in experimental colitis.

Then, how about the absorption of oral LF in non-infants or adults having no injury in the intestine? This is an important subject, because many of the anti-infectious effects of oral LF have been found in adult animals and humans without intestinal damage. In order to investigate these subjects, we analyzed the absorption of bovine LF or its fragments in the portal blood of healthy adult rats by several methods.

We attempted to detect the absorption of bovine LF by ELISA in comparison with ovalbumin (OVA). In rats fed a 4% solution of OVA, OVA was detected in the portal blood plasma, although the amount was low. When we added 5% glucose to the drinking water to increase the intestinal permeability by affecting the tight junctions, an increased level of OVA was detected. On the other hand, LF was not detected in the plasma of LF-fed rats in either the normal or glucose-treated condition. These results suggest that oral LF is less effective in permeating the intestine than OVA.

The analysis of oral LF in the portal blood of rats was performed using another method, SELDI affinity mass spectrometry with antibody capture. After OVA administration, OVA fragments with various molecular weights were detected in the portal blood plasma. In contrast, no LF fragments were detected in rats given oral bovine LF.

Finally, lactoferricin B (LFcin B)-containing fragments in the portal blood, including blood cells, were analyzed by SELDI affinity mass spectrometry with carboxymethyl capture and pepsin proteolysis. After a single dose or arbitrary administration of bovine LF, LFcin-containing fragments were not detected.

Based on these results, we can conclude that neither orally administered bovine LF nor its large molecular fragments are absorbed from the intestines of normal adult rats. Thus, it may not be reasonable to think that LF fragments are absorbed from the intestine and exert protective effects at the site of infection.

Host-protective mechanism of ingested LF

How then does oral LF enhance host defenses against pathogens without being absorbed? In other words, does oral LF modulate host immunity against infec-

tions? Based on the observations made in previous investigations (Table 1), we propose the following hypothesis. When LF is ingested, it is digested to some extent in the gastro-intestinal tract. Undigested LF or the digested fragments may bind to the epithelial cells of the gastro-intestine and modulate the intestinal mucosal immunity. Then the systemic or peripheral immunity is modulated through a network of mucosal and systemic immunity. Finally, the modulated systemic immunity enhances the host defense against infections.

The influence of oral LF on the mucosal immunity in the intestine has been reported by Tsuda's group. They reported that oral administration of bovine LF enhanced the production of IL-18 in the intestinal epithelial cells and IFN-γ in the lamina propria, and increased the number of $CD4^+$, $CD8^+$, and NK cells in the intestinal mucosa of both tumor-bearing mice and normal mice (Kuhara *et al.* 2000, Wang *et al.* 2000).

Currently, we are investigating the influence of oral bovine LF on systemic or peripheral immunity. Recently we reported the following results. We investigated the activity of splenic mononuclear cells of guinea pigs immunized with inactivated conidia of *Trichophyton mentagrophytes*, a filamentous fungus (Wakabayashi *et al.* 2002). Immediately after the immunization, LF administration was started. Seven days after the immunization, the proliferative response of splenocytes was enhanced by LF administration. The *Trichophyton*-killing activity of macrophages was enhanced by the addition of the splenocyte culture supernatant. The degree of enhancement was higher in the LF-fed animals. This result suggests that oral LF enhances humoral factor secretion from splenocytes, resulting in increased fungi-killing activity of macrophages.

Next, we reported the activities of peritoneal macrophages in mice intraperitoneally injected with inactivated *Candida albicans* (Wakabayashi *et al.* 2003). Immediately after *Candida* injection, LF administration was started. NO production by macrophages was induced by *Candida* priming and was enhanced by LF administration 7 days after the injection. *Candida* growth *in vitro* was inhibited by the macrophages and the inhibition was significantly enhanced in LF-fed animals.

More recently, in an oral candidiasis model, the cervical lymph node cells of mice were analyzed. The cell number and production of cytokines (IFN-γ and TNF-α) were significantly higher in LF-fed animals 6 days after the infection. Cytokine production by splenocytes of mice cutaneously infected with herpes simplex virus type-1 (HSV-1) was examined. The time course of IFN-γ production showed that the production was higher in LF-fed animals and was significant on day 5. IL-12 production was also increased on day 5 by LF administration. Oral LF attenuated the decreases of both body weight and the number of splenocytes in HSV-1-infected mice. The IFN-γ level was correlated with the body weight change, and the IL-12 level was correlated with the splenocyte number.

Conclusions

From these observations, we conclude that oral LF enhances systemic or peripheral immune responses to pathogens or their components as well as mucosal immunity in the intestines of animals. These enhanced immune responses may contribute to eradication of the pathogens, reduction of the symptoms, and maintenance of the homeostasis during infectious diseases.

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