Lactoferrin researchers descend on Nagoya Castle

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INTRODUCTION

Lactoferrin researchers descend on Nagoya Castle[1]

David B. Alexander, Hans J. Vogel, and Hiroyuki Tsuda

Lactoferrin is a member of the transferrin superfamily of proteins (see Vogel 2012 for a brief but comprehensive introduction to lactoferrin). Members of the transferrin superfamily are single-chain, glycosylated proteins that arose from a single common ancestor with iron-binding capability (Bai et al. 2016). The transferrins are an ancient protein family, and they are ubiquitous in metazoans (Lambert 2012; Bai et al. 2016). Gene duplication events, gene loss, and horizontal gene transfer have produced the variety of proteins that currently make up the transferrin superfamily (Lambert et al. 2005; Lambert 2012; Hughes and Friedman 2014; Bai et al. 2016). It is of considerable interest that many of these proteins have immunological functions (Geiser and Winzerling 2012; Lambert 2012; Mohd-Padil et al. 2013). One of the early gene duplication events, occurring sometime before the protosome/deuterostome split between 670 and 976 million years ago, was an internal duplication of the ancestral vertebrate transferrin gene, resulting in tandem genes that fused to generate a bilobal protein (Lambert et al. 2005; Bai et al. 2016). Interestingly, this duplication event allowed the evolutionary descendants of this new bilobed transferrin to exist as free proteins in the blood of mammals, as intact transferrins are not secreted by the kidney, but their isolated N- and C-terminal half-molecules are rapidly excreted in the urine (Williams et al. 1982).

The three groups of proteins that are most closely related to the well-known serum transferrin of vertebrates are melanotransferrin, lactoferrin (also known as lactotransferrin), and the inhibitor of carbonic anhydrase, which is a pseudogene in primates (Lambert et al. 2005; Lambert 2012). Melanotransferrins are thought to be the transferrin family members that are most closely related to the ancestral vertebrate transferrin and they are expressed in all major vertebrate groups (Lambert et al. 2005; Lambert 2012; Bai et al. 2016). While the present-day function of melanotransferrin is not known, this protein has the interesting property of being able to readily cross the blood–brain barrier (Demeule et al. 2002; Abbott et al. 2010). In reptiles and birds, differential glycosylation of transferrin in the liver and the oviduct results in the generation of two distinct types of transferrin: the transferrin that is found in the serum (which binds and transports iron), and ovotransferrin (a protein that exerts antimicrobial activity in the egg) (Lambert et al. 2005; Giansanti et al. 2012; Lambert 2012). In placental mammals, duplication of the transferrin gene after the divergence of placental mammals from marsupials generated separate transferrin and lactoferrin genes (Hughes and Friedman 2014), allowing the lactoferrin gene to evolve independently of the iron-transport function of serum transferrin.

A more recent evolutionary event was the generation of a second promoter in the lactoferrin gene (Siebert and Huang 1997; Liu et al. 2003). Transcription of lactoferrin from this promoter produces a lactoferrin protein known as delta-lactoferrin, which does not contain a signal peptide. Consequently, this particular lactoferrin is cytoplasmic and as a result of the nuclear localization signals contained in the lactoferrin sequence, delta-lactoferrin is translocated into the nucleus, where it acts as a transcription factor (Mariller et al. 2012).

In mature humans, lactoferrin (Lf) is expressed primarily at sites of contact with exogenous microbes: tear film, the upper respiratory tract, seminal fluid (Lf helps to protect the sperm from infection by the vaginal microbiota), and the specific granules of neutrophils (neutrophils deposit Lf at sites of microbial invasion) (Alexander et al. 2012). Consistent with this localization, human Lf exerts potent antimicrobial activity (Valenti and Antonini 2005; Berlutti et al. 2011; Alexander et al. 2012). Lf is also present at high levels in milk and helps protect the infant from infectious microbes. In addition to antimicrobial activity, Lf is thought to stimulate wound healing (Tang et al. 2010; Takayama and Aoki 2012). Consequently, Lf has important roles in the barrier function of the skin and mucosa.

Importantly, the interactions of Lf with microbes in the mucosa are not always negative. For example, bacteria from the *Neisseriaceae* and *Moraxellaceae* families can utilize Lf as an iron source. Thus, Lf can help modulate the resident microflora through both positive as well as negative interactions. In this issue, Ostan et al. (2017) report results from comparative studies on transferrin binding protein B (TbpB) and lactoferrin binding protein B (LbpB), giving insight into Lf and niche specificity of resident bacteria.

Lf in milk also helps to modulate the intestinal microbiome of the breastfeeding infant, enhancing the establishment of populations of some microbes and protecting against infections by others. Consequently, the levels of Lf in the milk can have serious consequences for the health of the breastfeeding infant. Villavicencio et al. (2017), have extensively reviewed the factors that affect lactoferrin concentration in human milk and colostrum. In another report, Ochoa and Sizonenko (2017) review the functions of Lf in neonates and preterm infants, including roles in the barrier function of the intestinal mucosa, modulation of the intestinal microbiome, and effects of Lf on the developing brain. Notably, Lf, like melanotransferrin and transferrin, readily crosses the blood–brain barrier (Abbott et al. 2010). These authors also briefly describe possible protective effects of Lf against other diseases related to premature birth, as well as the possible prevention of preterm delivery.

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[1]This Introduction is part of a Special Issue from the XIIth Lactoferrin Conference.

Fig. 1. The XIIth lactoferrin conference was held in the Fall of 2015, in a hotel venue that was adjacent to Nagoya Castle. While the original castle was built to ward off unwanted human invasions, the lactoferrin protein has a similar function by playing a protective role in the host defense immune system. [Colour online.]

Two papers included in this issue directly address the benefits of Lf in preventing preterm delivery. Otsuki and Imai (2017) report that treatment of 6 women at high risk of preterm delivery with bovine Lf (bLf) significantly improved the vaginal microflora, and Sessa et al. (2017) report that intravaginal administration of bLf resolved asymptomatic *Chlamydia trachomatis* in 6 of 7 pregnant women. All of the women treated with bLf in both studies had normal deliveries.

In the two studies noted above, it is notable that bovine Lf is protective in humans and is protective in a location where Lf is not normally present at high levels. However, given the antimicrobial function of lactoferrins throughout the Eutheria and the antimicrobial activity of a number of members of the transferrin superfamily throughout the animal kingdom, this protective function is not unexpected. Another study included in this issue reports that Lf is protective in the lower respiratory tract, another location where it is not normally present at high levels. In this study, Valenti et al. (2017) report on the effects of bLf treatment in a mouse model of lung infection by *Pseudomonas aeruginosa*, a pathogenic bacteria associated mostly with cystic fibrosis.

Applications of the antimicrobial activity of Lf do not have to be confined to the mucosal surface. Tomita et al. (2017) report on the amoebicidal effects of bLf on *Acanthamoeba*, an organism that causes severe keratitis. This amoebicidal activity suggests that bLf could potentially be used as an additive to solutions for disinfecting, rinsing, cleansing, and storing soft contact lens.

The protective function of Lf extends to direct interactions with barrier-forming cells. Four groups investigated the effects of this interaction. Tsou et al. (In press) reported that bLf attenuates lipopolysaccharide-induced inflammation by interacting with SPLUNC1 in a human nasal epithelial cell line. This capability could prove beneficial to patients with recurrent sinusitis. Shin et al. (2017) reported that bLf promotes the production of interferon lambda by the human intestinal cell line HT-29. Takayama et al. (2017) reported that bLf binds to the CXC cytokine receptor 4 and in doing so activates PI3K–Akt signaling in human intestinal and keratinocyte cell lines. Uchida et al. (2017) reported that bLf enhances human keratinocyte differentiation and the barrier function of skin.

In addition to clinical applications of Lf in humans, Lf also has important protective capability in economically valuable livestock. In this issue, Shimazaki and Kawai (2017) review the role of bLf in the treatment of bovine mastitis in dairy cows.

The biological activities of Lf-derived peptides form another emerging area of Lf research. Various Lf-derived peptides are well known as microbicidal agents (Arias et al. 2014), and the microbicidal activity of Lf-derived peptides is the subject of two reports in this special edition. Reyes-Cortes et al. (2017) investigated the microbicidal activity of Lf-derived peptides toward enteroaggregative *Escherichia coli*, while Aguilar-Diaz et al. (2017) investigated the microbicidal activity of Lf-derived peptides toward *Giardia intestinalis*. These organisms are widespread agents of disease, and the use of Lf-derived peptides offers the potential for non-antibiotic therapy.

Intriguingly, a number of cancer cells are also sensitive to host defense antimicrobial peptides (Papo and Shai 2005; Hoskin and Ramamoorthy 2008; Gaspar et al. 2013), and this class of peptides includes various Lf-derived peptides (Arias et al. 2014). In this issue, Arias et al. (2017) report the results of their investigation into the anticancer activities of bLf and hLF derived peptides against the Jurkat T-leukemia cell line. They also analyzed the properties of chimeric peptides designed to better penetrate cancer cells. In another study, Jiang and Lönnerdal (2017) report that bovine lactoferricins (an antimicrobial Lf-derived peptide) and bLf itself exert antitumor activity in the human colorectal cancer cell line HT-29, and they describe the signaling pathways that are activated by the lactoferricins and by bLf. These authors hypothesize that intact bLf may be internalized by HT-29 cells and directly activate gene transcription. As noted above, delta-Lf has a nuclear localization signal and is an active transcription factor; consequently, since the LF sequence contains the same nuclear localization signal, if LF is internalized into the cytoplasm of a cell it will be translocated into the nucleus and induce gene transcription. Indeed, in a separate study, also included in this issue, Jiang et al. (2017) report that Lf and Lf-sophorolipid complexes can be internalized by dermal fibroblasts and directly regulate gene transcription. Notably, the gene transcription profiles described in this study supply

additional evidence that Lf is beneficial to skin health and promotes the barrier function of the skin.

The interaction of Lf with cells that are not normally associated with Lf-mediated activity can have effects other than the protective functions described in the reports listed above. Ishii et al. (2017) report that bLf down-regulates microphthalmia-associated transcription factor in melanin-producing cells in vitro, leading to suppression of melanin production. Delivery of molecules to target sites, such as to melanocytes lying below the stratum corneum of the epidermis, can be greatly enhanced by interaction of the molecule with sophorolipids, and accordingly Matsumiya et al. (2017) have carefully analyzed the interactions between bLf and sophorolipids.

Ingestion is a common method of self-administration of Lf. However, the effects of ingested Lf are complicated by the fact that Lf is digested in the mature gastrointestinal tract, and gastric digestion of lactoferrin generates Lf-derived peptides (Kuwata et al. 1998*a*, 1998*b*). Furthermore, ingestion of smaller amounts of Lf results in more complete digestion of Lf than ingestion of large amounts of Lf or administration of Lf by oral gavage. Alexander et al. (2017) report a case study of a patient with Crohn's disease in remission, who has been ingesting bLf daily for 4.5 years. The authors theorize that the effects of bLf on the remissive state of the patient's Crohn's disease is primarily or wholly due to the activity of Lf-derived peptides. In another study, Nakamura et al. (2017) fed rats a diet containing 5% bLf. In these rats, the addition of bLf to a high-cholesterol diet resulted in increased fecal cholesterol and decreased hepatic cholesterol levels. In a third study, Hwang et al. (2017) administered human or mouse recombinant Lf to mice by oral gavage. They report that administration of Lf reduced *Mycobacterium tuberculosis* induced lung pathology.

Another type of administration is intraperitoneal injection of Lf. This type of administration is designed to increase the level of Lf in the blood. Maekawa et al. (2017) report that intraperitoneal injection of bLf enhances glucose absorption from the small intestine and elevates plasma insulin, leading to a decrease in plasma glucose.

The Lf field is maturing, and as can be seen from the papers presented at the XIIth Lactoferrin conference in Nagoya, in 2015; the research emphasis is increasingly being directed towards the development of practical applications of Lf and Lf-derived peptides. The final paper in this issue is a review of recent progress in Lf and Lf-derived peptide based research and development of applications in China by Wang et al. (2017). It will be interesting to see if the trend towards more practical applications of Lf and its peptides will continue during the XIIIth Lactoferrin conference, which will be held in late 2017, in Rome, Italy.

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