Ingested soluble CD14 from milk is transferred intact into the blood of newborn rats

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Abstract

Background—Milk contains immunological constituents that comprise an edible immune system conveyed from mother to newborn. Soluble Cluster of Differentiation 14 (sCD14) is a protein found in significant quantities in human milk (~8–29 μg/ml). At a tenfold lower concentration in the blood (~3 μg/ml), the most notable role of sCD14 is to sequester lipopolysaccharide of Gram-negative bacteria from immune cells.

Methods—To explore the pharmacodynamics of this milk protein and its biological fate, the biodistribution of radiolabeled sCD14 (14C, 125I) was monitored in 10 d old rat pups.

Results—Up to 3.4 ± 2.2% of the radiolabeled-sCD14 administered was observed, intact, in the pup blood for up to 8 h post-ingestion. Additionally, 30.3 ± 13.0% of the radiolabeled-sCD14 administered was observed degraded in the stomach at 8 h post-ingestion. A reservoir of intact, administered sCD14 (3.2 ± 0.3%), however, remained in the stomach at 8 h post-ingestion. Intact sCD14 was observed in the small intestine at 5.5 ± 1.6% of the dose fed at 8h post-ingestion.

Conclusions—The presence of intact sCD14 in the blood and gastrointestinal tract of newborns post-ingestion has implications in the development of allergies, obesity and other inflammation-related pathogeneses later in life.

INTRODUCTION

Cluster of Differentiation 14 (CD14) is a 48 kDa pattern recognition receptor first discovered as a sensor for lipopolysaccharide (LPS) of Gram-negative bacteria. CD14 exists either as a GPI-anchored membrane protein (mCD14) on the cell surface or as a soluble...
protein (sCD14) found in bodily fluids. Soluble CD14 is observed in the blood at a concentration of 3.71 ± 0.59 μg/ml and at a fold-higher concentration in human milk, 20.10 ± 8.74 μg/ml (5 days postpartum) to 12.16 ± 3.75 μg/ml (3 months postpartum (1, 2). The two forms of CD14 (m or s) appear functionally interchangeable as they both can enhance proinflammatory signalling in response to LPS through Toll-like receptor 4 (TLR4), alerting the immune system of potential infections (3).

In blood, circulating sCD14 decreases LPS-related mortality and septic shock presumably by sequestering LPS from mCD14/TLR4-expressing immune cells (4). This allows clearance of LPS from the body before activation of the immune system. Recent studies have also implicated sCD14 in inflammation-related diseases. For example, both circulating and milk sCD14 levels have been correlated to fat mass in humans, and the genomic elimination of the CD14 gene in mice attenuated symptoms of obesity, such as hypertension (5–7). Furthermore, CD14 is thought to influence the type of bacteria colonizing the gastrointestinal (GI) tract of infants (8). Therefore, like many other immunologically relevant agents present in human milk, such as serum proteins, cytokines and immunoglobulins, milk-derived sCD14 may play a role in inflammation, development and overall infant health as discussed in a recent review (9).

The high concentration of sCD14 in human milk exposes a breastfeeding infant to milligram quantities of the protein per day, however, in our initial study, neither intact nor degraded portions of sCD14 are found in the feces of breastfed infants (10). Immunoprecipitations of sCD14 from milk and in vitro digests demonstrate that sCD14 is able to complex with other milk proteins, namely alpha-lactalbumin, which protect it from degradation (11). Taken together, the combined proteolytic protection of sCD14 by milk components and lack of sCD14 in infant feces raise the possibility that sCD14 may be absorbed intact along the GI tract of the infant, as we earlier suggested (10).

Whole protein uptake across the epithelium and into the blood stream has been previously described for other milk proteins such as immunoglobulins (12). Once translocated to the blood, these milk proteins contribute to the infant’s endogenous serum pool of proteins, stimulating the immune system and offering passive immunity (13, review). Because sCD14 levels continue to increase during the first 18 months of life, sCD14 provided by the mother via her milk may afford additional surveillance against bacteria in the GI tract or blood of the infant (8).

In healthy, full-term infants ‘gut closure’ (a decrease in intestinal permeability with age) occurs within a few days postpartum, which can be altered depending on the nutrient source (human milk versus formula (14). In rodents, gut closure is further delayed and correlates to the weaning age of 17–21 days postpartum (15). In this present study, 10 d old rat pups were used as a model for newborn human infants in which gut closure has not yet occurred (term infants 1–3 d old or preterm infants 1–10 d old). This age was chosen as it correlates to the greatest expression of sCD14 in human milk, which can reach concentrations as high as 67.09 ± 27.61 μg/ml in colostrum (1, 2). Using radiolabeled proteins as a means to track digestive fate, we address the hypothesis that sCD14 remains intact along the digestive tract and is absorbed intact into the circulatory system, post-ingestion.
RESULTS

Ingested sCD14 persists in the GI tract

Within the stomach, 3.2 ± 0.3% of the [14C]CD14 dose fed remained intact at 8 h post-ingestion, which did not significantly differ from the amount at 0.3 h post-ingestion (3.5 ± 1.3%, P=0.838, Figure 1A). The amount of degraded [14C]CD14 at 0.3 h post-ingestion (50.0 ± 17.9%) also did not significantly decrease over the duration of the experiment (30.3 ± 13.0% at 8 h, P=0.423). This differs from the digestible control protein, BSA. In the stomach, 7.7 ± 2.9% of the [14C]BSA dose fed was present intact at 0.3 h, which significantly decreased to 1.3 ± 0.5% by 8 h post-ingestion (P=0.027, Figure 1A). The stomach also contained no more than 0.3 ± 0.1% of the dose fed of degraded [14C]BSA at all time points.

In the duodenum, no more than 2.5 ± 0.7 % of the [14C]CD14 dose fed, degraded or intact, was observed at any time point, likely due to the small size of the tissue (Figure 1B). A similar trend was observed for ingested [14C]BSA, with no more than 0.5 ± 0.5% present in the duodenum, degraded or intact, at any time point (Figure 1B). In the jejunum, however, the amount of intact [14C]CD14 increased from 0.9 ± 0.3% of the dose fed at 0.3 h to 5.5 ± 1.6% of the dose fed at 8 h (P=0.045, Figure 1C). Similarly, the amount of degraded [14C]CD14 in the jejunum increased from 9.6 ± 2.0% at 0.3 h to 37.5 ± 9.8% of the dose fed at 8 h post-ingestion (P=0.049). The control protein, BSA, showed an opposite trend (Figure 1C). In the jejunum, the amount at 0.3 h for both intact and degraded BSA (4.4 ± 1.0% and 8.3 ± 2.2%, respectively) significantly decreased by 8 h (0.8 ± 0.1% and 0.8 ± 0.2%, respectively; P=0.024, P=0.027).

Similar to the jejunum, the ileum and large intestine demonstrated an increase in both intact and degraded [14C]CD14 from 0.3 to 8 h post-ingestion. For example, in the large intestine the 0.05 ± 0.01% of the dose fed of intact [14C]CD14 significantly increased to 0.3 ± 0.1% of the dose fed at 8 h post-ingestion (P= 0.042, Figure 1E). The amount of intact BSA, however, decreased in the ileum over time (0.2 ± 0.09% to 0.05 ± 0.03% of the dose fed, from 0.3 to 8 hr, P=0.045), and the amount of BSA in the large intestine showed no significant change across time points (Figure 1E).

Ingested sCD14 is transferred to the blood

Intact (48 kDa) and degraded [125I]sCD14 (30 kDa and 25 kDa) were observed in the stomach for up to 8 h (Figure 2A). By exaggerating the image contrast, intact [125I]sCD14 was also observed in the jejunum for up to 8 h post-gavage (Supplemental Figure S1 (online)). No [125I]sCD14 was detected in the duodenum or ileum, likely due to the small size of tissue and relative protein content (Supplemental Figure S2 (online)). Most interestingly, intact [125I]sCD14 (48 kDa) was detected in the blood at 4 h post-ingestion and persisted up to 8 h post-ingestion (Figure 2A). In comparison, both intact (66 kDa) and degraded [125I]BSA (50, 40 and 30 kDa) were observed in the stomach for up to 8 h (Figure 2B). [125I]BSA was observed in both intact and in multiple degraded forms (50, 40 and 30 kDa) in the duodenum, jejunum and ileum samples. Neither intact nor degraded [125I]BSA was detected in the blood at any of the sampling times (Figure 2B).
In a separate experiment, intact $^{125}$I-sCD14 was detected in the blood as early as 0.3 h post-gavage (0.5 ± 0.4% of the dose fed), which significantly increased to 2.5 ± 1.2% of the dose fed by 1 h post-gavage ($P=0.021$, Figure 3A). The percentage of intact $^{125}$I-sCD14 in the blood did not significantly change after 1 h (3.4 ± 2.2% of the dose fed at 8 h, Figure 3). Degraded $^{125}$I-sCD14 was also observed in the blood by 0.3 h post-ingestion, at a concentration of 1.08 ± 0.5% of the dose fed. The concentration of degraded $^{125}$I-sCD14 in the blood at 0.3 h significantly increased at 4 h post-ingestion ($P=0.024$), at which time it reached 4.9 ± 2.0% of the dose fed (Figure 3B).

Using SDS-PAGE and phosphor-imaging, the circulating $^{125}$I-sCD14 was confirmed to be fully intact (48 kDa, Figure 4A). Degraded sCD14 was not detected by these methods, likely due to its small size (<10 kDa). This was confirmed by silver staining the SDS-PAGE gels of the >30 kDa or <30 kDa size-fractionated samples, where no protein was observed in the <30 kDa fraction (Figure 4B).

**Rat milk contains sCD14**

Because the pups were ingesting rat milk from their mother pre- and post-gavage, any unlabeled rat-sCD14 within the milk may have hindered labeled-CD14 uptake across the GI tract. A western blot using milk clots from pups fed PBS only showed the presence of sCD14 in rat milk (Figure 5).

**DISCUSSION**

The bioactive components of human milk that promote development and provide protection to the infant, including the bacterial sensor sCD14, must be proteolytically resistant to digestion or form complexes with other proteins to avoid degradation and retain functionality in the infant’s GI tract or circulatory system. Secretory IgA and lactoferrin are well known examples of bioactive proteins that survive passage through the infant’s digestive system (10, 16). Likewise, in this study we show that sCD14 was able to evade digestion in the GI tract as, 3.2 ± 0.3% of ingested $^{14}$C-CD14 remained undigested in the stomach for up to 8 h. In comparison, the digestible control, BSA, decreased from 7.7 ± 2.9% at 0.3 h to 1.3 ± 0.5% of the dose fed at 8 h post-ingestion (Figure 1A). Soluble CD14 was also able to enter and persist in the small intestine, as 5.5 ± 1.6% of the dose fed of sCD14 was observed intact in the jejunum at 8 h post-ingestion (Figure 1C). This persistence may be due, in part, to the interaction of sCD14 with other milk proteins. For example, we previously showed alpha-lactalbumin to interact with sCD14 in milk and protect it from digestion in vitro (11). Rat milk, which was ingested pre- and post-gavage by the pups, contains alpha-lactalbumin at a concentration of 1.5 g/L at 8 days post-parturition which increases to 3 g/L at 15 days post-parturition (17). In vivo, alpha-lactalbumin in rat milk likely confers the same proteolytic protection of sCD14 observed in vitro.

The high percentage of degraded sCD14 observed in all organs using liquid scintillation counting (Figure 1, white bars) was not observed by SDS-PAGE and phosphor-imaging (Figure 2A). This is likely due to the small size of the peptides produced during digestion (<10 kDa), which would migrate off the gel, limiting detection. Previous in vitro work showed human milk sCD14 to be partly resistant to pepsin digestion, which was confirmed.
herein through the observation of intact sCD14 in the stomach for up to 8 h post-ingestion (Figure 2A, 10). Additionally, in vitro pepsin and pancreatin digestion, simulating the small intestine, showed human milk-sCD14 to be proteolytically sensitive, and produced fragments of approximately 20 kDa (10). In this present report, [14C}sCD14 <30 kDa was detected in the small intestine at all time points (Figure 1B–D), but these fragments were not detected using phosphor-imaging (Figure 2). This may be due to the location of the radio-label along the peptide chain, which may have prevented detection of some fragments depending on how the protein was digested.

Whether intact or degraded, ingested sCD14 may be acting as an important regulator of development in the infant GI tract. Intact sCD14 in the GI tract may aid in dampening the immune response to the influx of colonizing bacteria as the child develops just as sCD14 is able to neutralize bacterial LPS in the blood (4, 18). Similarly, degraded sCD14 may provide protection to the infant, as sCD14 peptides as small as 20 amino acids (residues 81–100 especially) have been shown to contain effective LPS neutralizing capabilities (19). Furthermore, a fragment of CD14 (residues 139–160) has been shown to protect human lymphocytes from gliotoxin-induced death, and sCD14 has been shown to be a survival factor for leukemic cells (20, 21). If sCD14 behaves similarly along the GI tract of infants, it may be promoting gut closure and intestinal epithelial cell homeostasis. In support of this, it has been shown that digested infant formulas, which notably lack sCD14, increase death of intestinal cells, whereas digested human milk preserves their function (22).

Strikingly, sCD14 was detected intact in the blood at 4 h post-gavage, where it remained for up to 8 h (Figure 2A). This finding was confirmed by a second experiment in which the blood of gavage-fed pups was sampled each hour following sCD14 ingestion (Figures 3A and 4A). We found that ~3% of the ingested sCD14 was absorbed intact into the blood, which did not significantly change up to 8 h post-ingestion (Figure 3A). When extrapolated, this amount may represent up to 30% of a newborn’s circulating sCD14. As more studies describing the constituents of milk that are able to cross the tight infant epithelial barrier are published, the notion of ‘you are what you eat’ gains validity. The transfer of beneficial immune components, such as the sCD14 reported here, immunoglobulins, and non-inherited maternal antigens can positively affect the infant’s health by conferring additional immunity (23, 24). For example, once ingested, noninherited maternal antigens present in the mother’s milk convey tolerance to the infant during bone marrow transplantation later in life (24). This type of donated protection via milk into the infant’s blood is likely conserved across other immune proteins, such as sCD14.

Whole protein uptake of milk components has been well described for immunoglobulins and lactoferrin, which are receptor mediated and conserved across species including humans and rodents (12, 25). One potential route of sCD14 uptake may involve the bacterial recognition receptor TLR4, which is present on multiple gut-associated cell types including intestinal epithelial cells of adults and newborns (26). TLR4 interacts with both mCD14 and sCD14 to signal the presence of bacteria and alert the immune system. In vitro studies have shown that TLR4 internalization during LPS exposure is CD14-dependent in B cells, and translocation of LPS and whole bacteria by intestinal epithelial cells has been demonstrated in vitro and in vivo (27, 28). This internalization of LPS and whole bacteria may represent a potential
mechanism for sCD14 uptake in vivo along the GI tract, as sCD14 may ‘piggyback’ on luminal LPS while the latter is being translocated. If the uptake of sCD14 is specific and receptor mediated, the 3.4 ± 2.2% of the intact $^{125}$I-sCD14 fed that is translocated to the blood at 8 h may be an underrepresentation of the total amount of sCD14 absorbed in the GI tract (Figure 3A). Because rat milk contains endogenous rat-sCD14 (Figure 5), unlabeled endogenous-protein may be competing for the uptake mechanisms used by radiolabeled sCD14, thereby hindering labeled-protein uptake. Consequently, further experiments to better quantify the total amount of sCD14 translocated to the blood are warranted.

In the blood, the classical role of circulating sCD14 is to attenuate bacterial-induced immune responses by sequestering bacterial components, including LPS, from immune cells and protecting against exaggerated immune responses such as septic shock (4). The exact role of ingested sCD14 in the infant has yet to be determined, but the molecule’s newly found associations with inflammation-related diseases suggest its transfer from mother to infant holds significance. For example, there are numerous situations where removing both mCD14 and sCD14 expression results in attenuation of inflammation-related disease phenotypes. In obesity there is a chronic low-grade state of inflammation which is affected by the presence of CD14. When the CD14 coding region was removed from the genome, mice still possessed the ability to become obese but they gained less fat compared to wild type mice and suffered from less obesity-related phenotypes, such as hypertension (6). Another closely related example is the effect of CD14 on insulin resistance. Specifically, when wild type mice were given a bone marrow transplant with CD14 null cells, they no longer became glucose intolerant or insulin resistant when fed a high fat diet (7). Therefore, sCD14 levels may impact inflammatory responses in the infant.

In the context of LPS-response, elevated levels of sCD14 were able to attenuate exaggerated immune responses in mice in otherwise normal physiological contexts (18). Perhaps the presence of additional sCD14, obtained from the mother’s milk, provides similar protection for the infant during LPS response, as well as in other systems. For example, delivering additional recombinant sCD14 via injection to adult wild type mice was able to increase glucose tolerance and increased insulin action in the same fashion as a total CD14 knock out (7). Furthermore, the ability of additional sCD14 to ameliorate negative inflammatory symptoms was also seen in allergy development. It has been shown that children with allergies often have decreased levels of circulating sCD14, and that exogenous sCD14 was able to suppress allergen-induced Th2 differentiation in vitro (8, 29). The link between the level of sCD14 in mother’s milk and atopy and eczema development in the infant, however, remains controversial (30–32).

Therefore, the significance of the transfer of sCD14 from human milk into the circulation of the infant should not be underestimated. As new roles of sCD14 in disease pathogenesis are uncovered, the protein’s impact on infant development may become clearer. Current work includes the use of CD14 null mice to determine the unhindered quantity of ingested sCD14 transferred to the infant’s blood and the functionality of ingested sCD14 in vivo by testing its capability to detect LPS. Future studies to determine the benefit of sCD14 ingestion in the infant in regards to other systems, such as GI colonization, obesity, diabetes and allergy are imperative.
METHODS

Radiolabeling of proteins

Human recombinant sCD14 (R&D Systems, Minneapolis, MN) and BSA (Sigma-Aldrich, Oakville, ON, Canada) were labeled with $^{14}$CCH$_3$I using 52.9 mCi/mmol (sCD14) or 10 mCi/mmol (BSA) (Perkin Elmer, Waltham, MA and Sigma-Aldrich). Proteins (100 μg of sCD14 or 500 μg of BSA) were lyophilized and labeled using our previously established protocol (33).

Separately, sCD14 and BSA were labeled with $^{125}$I NaI (MP Biomedical, Solon, OH) using Iodination Beads (Thermo Scientific, Rockford, IL) following the manufacturer’s protocol with 100 μg of protein and 1 mCi of $^{125}$I NaI in a total volume of 1.1 ml PBS. Proteins were recovered by centrifugation at 1000 × g for 2 min using a 2 ml Zeba Desalt spin column (Thermo Scientific). The resultant specific radioactivities were as follows: $^{14}$CsCD14, 1.63 × 10^5 disintegrations per minute (dpm)/μg; $^{14}$CBSA, 3.44 × 10^4 dpm/μg; $^{125}$IsCD14, 12.16 × 10^6 dpm/μg; $^{125}$IBSA, 13.7 × 10^6 dpm/μg. Radiolabeled proteins were stored at −70°C.

Feeding studies and protein isolation

Feeding studies were conducted in accordance with the University of Ottawa’s Animal Care and Veterinary Service under approval permit ID number ‘BMI 77’ and approved by the University of Ottawa’s Animal Care Committee. Sprague-Dawley rat pups aged 10 d old were used as a model of pre-weaning infants as at this age the animals only ingest their mothers’ milk. In total, 26 pups (derived from three litters of 13 pups each) weighing between 11 g and 26 g were fasted for 2 h at 37°C to partially empty the stomach and accommodate for the gavage protein solution (34). Pups were gavage fed 25 μg in 250 μL of PBS of either $^{14}$CsCD14, $^{14}$CBSA, $^{125}$IsCD14 or $^{125}$IBSA using our previous technique (34). As a control, one pup per experiment was fed PBS alone (data not shown). BSA was chosen as a digestible control because it is known to be easily degraded and absorbed in the GI tract (35). Animals were anaesthetized with isoflurane at 0.3, 4 and 8 h post-gavage and euthanized by cardiac puncture. Organs were harvested, flash frozen in liquid nitrogen and stored at −70°C. The GI tract was separated into the duodenum, jejunum, ileum and large intestine by length (7%, 80%, 2% and 11%, respectively, 36).

To determine the amount of sCD14 transferred to the blood, five Sprague-Dawley pups (10 d old) were fasted as described above and gavage fed 25 μg of $^{125}$IsCD14 or PBS alone (data not shown) and returned to their mother. Blood samples (10 μL) were collected from the hind leg by needle prick for up to 8 h post-gavage and stored at −70°C.

Quantifying intact and degraded proteins

Organs and luminal contents were weighed and homogenized on ice in 150 μL buffer (50 mM Tris-HCl pH 7.4, 2 mM EDTA, 150 mM NaCl, 0.5 mM DTT and protease inhibitor cocktail, Sigma-Aldrich) using a micropestle (Eppendorf, Westbury, NY). Samples were sonicated on ice three times using a 30 sec on/off cycle at 5 W and cell debris was removed by centrifugation. Samples were pre-filtered using a 0.22 μm spin filter (Amicon, Millipore,
Bedford, MA), followed by separation through a 30,000 molecular weight cut-off spin filter (Amicon). Flow-throughs (<30 kDa) and retentates (>30 kDa) were added to 5 ml of liquid scintillation cocktail (ScintiSafe-Econo1, Fisher Scientific, Ottawa, ON, Canada) and radioactivities were quantified using a Tri-Carb liquid scintillation counter (Perkin Elmer).

**Determining protein intactness**
Isolated proteins were subject to size separation by SDS-PAGE using AnyKD SDS-polyacrylamide gradient gels (Bio-Rad, Mississauga, ON, Canada) and electrophoresis. Gels were silver stained, exposed to a storage phosphor screen (GE Healthcare, Piscataway, NJ) and imaged using a Typhoon Trio Imager (GE Healthcare).

**Detecting CD14 in rat milk**
From three pups fed PBS alone, stomach contents were resuspended in PBS and pooled. Proteins, including 100 ng of human recombinant CD14 (R&D Systems) as a positive control and a biotinylated molecular weight marker (7727, Cell Signalling Technology, Inc., Danvers, MA) were size separated using SDS-PAGE and transferred to nitrocellulose membrane. A mouse anti-human CD14 antibody (cross reactive to rat CD14, MAB 3831, R&D Systems), a goat anti-mouse IgG horse radish peroxidase (HRP)-linked antibody (HAF007, R&D Systems), and a goat anti-biotin HRP-linked antibody to recognize the molecular weight markers (7727, Cell Signalling Technology, Inc., Danvers, MA) were used in a western blot of the milk proteins. Proteins were visualized using exposure to enhanced chemiluminescent (ECL) substrate (GE Healthcare).

**Data Analysis**
Percent dose was calculated using the formula [(dpm/g organ)x(total organ weight)]/dpm dose fed. Total organ weight of blood was calculated using the approximation that blood represents 7% of the body weight. Data were analyzed by 2-tailed paired or unpaired t-tests (Sigma Plot 12.1, Systat, San Jose, CA). An alpha level of <0.05 was considered significant.

**Supplementary Material**
Refer to Web version on PubMed Central for supplementary material.

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**References**
1. Labeta MO, Vidal K, Nores JE, et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. J Exp Med. 2000; 191:1807–1812. [PubMed: 10811873]
2. Vidal K, Labeta MO, Schiffrin EJ, Donnet-Hughes A. Soluble CD14 in human breast milk and its role in innate immune responses. Acta Odontol Scand. 2001; 59:330–334. [PubMed: 11680654]
3. Frey EA, Miller DS, Jahr TG, et al. Soluble CD14 participates in the response of cells to lipopolysaccharide. J Exp Med. 1992; 176:1665–1671. [PubMed: 1281215]
4. Kitchens RL, Thompson PA, Viriyakosol S, O’Keefe GE, Munford RS. Plasma CD14 decreases monocyte responses to LPS by transferring cell-bound LPS to plasma lipoproteins. J Clin Invest. 2001; 108:485–493. [PubMed: 11489942]
5. Collado MC, Laitinen K, Salminen S, Isolauri E. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. Pediatr Res. 2012; 72:77–85. [PubMed: 22453296]
6. Roncon-Albuquerque R, Moreira-Rodrigues M, Faria B, et al. Attenuation of the cardiovascular and metabolic complications of obesity in CD14 knockout mice. Life Sci. 2008; 83:502–510. [PubMed: 18761356]
7. Fernandez-Real JM, Perez del Pulgar S, Luche E, et al. CD14 modulates inflammation-driven insulin resistance. Diabetes. 2011; 60:2179–2186. [PubMed: 21700881]
8. Lundell AC, Adlerberth I, Lindberg E, et al. Increased levels of circulating soluble CD14 but not CD83 in infants are associated with early intestinal colonization with Staphylococcus aureus. Clin Exp Allergy. 2007; 37:62–71. [PubMed: 17210043]
9. Chatterton DE, Nguyen DN, Bering SB, Sangild PT. Anti-inflammatory mechanisms of bioactive milk proteins in the intestine of newborns. Int J Biochem Cell Biol. 2013; 45:1730–1747. [PubMed: 23660296]
10. Blais DR, Harrold J, Altosaar I. Killing the messenger in the nick of time: persistence of breast milk sCD14 in the neonatal gastrointestinal tract. Pediatr Res. 2006; 59:371–376. [PubMed: 16492974]
11. Spencer WJ, Binette A, Ward TL, et al. Alpha-lactalbumin in human milk alters the proteolytic degradation of soluble CD14 by forming a complex. Pediatr Res. 2010; 68:490–493. [PubMed: 20710700]
12. Ladinsky MS, Huey-Tubman KE, Bjorkman PJ. Electron tomography of late stages of FcRn-mediated antibody transcytosis in neonatal rat small intestine. Mol Biol Cell. 2012; 23:2537–2545. [PubMed: 22573886]
13. Kuo TT, Baker K, Yoshida M, Blumberg RS, et al. Neonatal Fc receptor: from immunity to therapeutics. J Clin Immunol. 2010; 30:777–789. [PubMed: 20886282]
14. Taylor SN, Basile LA, Ebeling M, Wagner CL. Intestinal permeability in preterm infants by feeding type: mother’s milk versus formula. Breastfeed Med. 2009; 4:13–17.
15. Teichberg S, Isolauri E, Wapnir RA, Roberts B, Lifshitz F. Development of the neonatal rat small intestinal barrier to nonspecific macromolecular absorption: effect of early weaning to artificial diets. Pediatr Res. 1990; 28:31–37. [PubMed: 2377394]
16. Goldman AS, Garza C, Schanler RJ, Goldblum RM. Molecular forms of lactoferrin in stool and urine from infants fed human milk. Pediatr Res. 1990; 27:252–255. [PubMed: 2320391]
17. Nicholas KR, Hartmann PE, McDonald BL. Alpha-Lactalbumin and lactose concentrations in rat milk during lactation. Biochem J. 1981; 194:149–154. [PubMed: 7309575]
18. Jacque B, Stephan K, Smirnova I, Kim B, Gilling D, Polorak A. Mice expressing high levels of soluble CD14 retain LPS in the circulation and are resistant to LPS-induced lethality. Eu J Immunol. 2006; 36:3007–3016.
19. Voss S, Welte S, Fotin-Mleczek M, et al. A CD14 domain with lipopolysaccharide-binding and -neutralizing activity. Chembiochem. 2006; 7:275–286. [PubMed: 1644757]
20. Tartakovsky B, Sredni B, Zigman-Hoffman E, Senyory G, Naparstek E. A peptide of CD14 protects human lymphocytes from gliotoxin-induced apoptosis. Int J Pept Res Ther. 2012; 18:249–258.
21. Seiffert M, Schulz A, Ohl S, Dohner H, Stilgenbauer S, Lichter P. Soluble CD14 is a novel monocyte-derived survival factor for chronic lymphocytic leukemia cells, which is induced by CLL cells in vitro and present at abnormally high levels in vivo. Blood. 2010; 116:4223–4230. [PubMed: 20660791]
22. Penn AH, Altshuler AE, Small JW, Taylor SF, Dobkins KR, Schmid-Schonbein GW. Digested formula but not digested fresh human milk causes death of intestinal cells in vitro: implications for necrotizing enterocolitis. Pediatr Res. 2012; 72:560–567. [PubMed: 23007028]

23. Weltzin R, Lucia-Jandris P, Michetti P, Fields BN, Kraehenbuhl JP, Neutra MR. Binding and transepithelial transport of immunoglobulins by intestinal M cells: demonstration using monoclonal IgA antibodies against enteric viral proteins. J Cell Biol. 1989; 108:1673–1685. [PubMed: 2541137]

24. Aoyama K, Koyama M, Matsuoka K, et al. Improved outcome of allogeneic bone marrow transplantation due to breastfeeding-induced tolerance to maternal antigens. Blood. 2009; 113:1829–1833. [PubMed: 19124834]

25. Lonnerdal B, Jiang R, Du X. Bovine lactoferrin can be taken up by the human intestinal lactoferrin receptor and exert bioactivities. J Pediatr Gastroenterol Nutr. 2011; 53:606–614. [PubMed: 21832946]

26. Vamadevan AS, Fukata M, Arnold ET, Thomas LS, Hsu D, Abreu MT. Regulation of Toll-like receptor 4-associated MD-2 in intestinal epithelial cells: a comprehensive analysis. Innate Immun. 2010; 16:93–103. [PubMed: 19710105]

27. Tanimura N, Saitoh S, Matsumoto F, Akashi-Takamura S, Miyake K. Roles for LPS-dependent interaction and relocation of TLR4 and TRAM in TRIF-signaling. Biochem Biophys Res Commun. 2008; 368:94–99. [PubMed: 18222170]

28. Neal MD, Leaphart C, Levy R, et al. Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. J Immunol. 2006; 176:3070–3079. [PubMed: 16493066]

29. Lundell AC, Andersson K, Josefsson E, Steinkasserer A, Rudin A. Soluble CD14 and CD83 from human neonatal antigen-presenting cells are inducible by commensal bacteria and suppress allergen-induced human neonatal Th2 differentiation. Infect Immun. 2007; 75:4097–4104. [PubMed: 17526743]

30. Jones CA, Holloway JA, Popplewell EJ, et al. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. J Allergy Clin Immunol. 2002; 109:858–866. [PubMed: 11994712]

31. Savilahti E, Siltanen M, Kajosaari M, Vaarala O, Saarinen KM. IgA antibodies, TGF-beta1 and -beta2, and soluble CD14 in the colostrum and development of atopy by age 4. Pediatr Res. 2005; 58:1300–1305. [PubMed: 16306212]

32. Ismail IH, Licciardi PV, Oppedisano F, Boyle RJ, Tang ML. Relationship between breast milk sCD14, TGF-beta1 and total IgA in the first month and development of eczema during infancy. Pediatr Allergy Immunol. 2013; 24:352–360. [PubMed: 23577672]

33. Davis LD, Spencer WJ, Pham VT, et al. (14)C radiolabeling of proteins to monitor biodistribution of ingested proteins. Anal Biochem. 2011; 410:57–61. [PubMed: 21094630]

34. Davis LD, Spencer WJ, Mack DR, Altosaar I. Maternal separation and gastrointestinal transit time in neonate rats. Lab Anim. 2011; 45:280–282. [PubMed: 21828078]

35. Eriksen EK, Holm H, Jensen E, et al. Different digestion of caprine whey proteins by human and porcine gastrointestinal enzymes. Br J Nutr. 2010; 104:374–381. [PubMed: 20307348]

36. DeSesso JM, Williams AL, John EM. Contrasting the gastrointestinal tracts of mammals: factors that influence absorption. Annu Rep Med Chem. 2008; 43:353–371.
Figure 1.
Biodistribution of intact (>30 kDa, black) and degraded (<30 kDa, white) $^{14}$C$sCD14$ and a digestible control protein, $^{14}$C$BSA$, post-ingestion in 10 d old rats. A, stomach; B, duodenum; C, jejunum; D, ileum; E, large intestine. Rat pups were gavage fed 25 μg of $^{14}$C$sCD14$ (1.63 ×10$^5$ dpm/μg) or $^{14}$C$BSA$ (3.44 ×10$^4$ dpm/μg). Post-euthanasia (0.3, 4 or 8 h), proteins were extracted from organs, size separated using a 30 kDa cut-off spin column and liquid scintillation counted. Data are presented as mean ± SD, and n=3 for each time point. *P<0.05, using a Student’s t-test.
Figure 2.
The biodistribution of $^{125}\text{I}\text{sCD14}$ (A) and a digestible control protein, $^{125}\text{I}\text{BSA}$ (B), post-ingestion in 10 d old rats. Rat pups were gavage fed 25 μg of $^{125}\text{I}\text{sCD14}$ (12.16 $\times 10^6$ dpm/μg) or $^{125}\text{I}\text{BSA}$ (13.7 $\times 10^6$ dpm/μg). Post-euthanasia (0.3, 4 or 8 h), proteins were extracted from harvested organs, size separated by SDS-PAGE and phosphor-imaged. Stomach (St) and contents (St Con), duodenum (Duo) and contents (Duo Con), jejunum (Je) and contents (Je Con), ileum and contents (Ileum Con), large intestine (L In) and contents (L In Con), liver and blood. Figure is representative of n=3.
Figure 3.
Uptake of intact (A, >30 kDa) and degraded (B, <30 kDa) $[^{125}\text{I}]$sCD14 into the blood of 10 d old rat pups. Data are presented as mean ± SD, and n=4. Percent dose fed >30 kDa (A) at 0.3 h was significantly less than all subsequent time points (*, $P<0.05$). Percent dose fed <30 kDa (B) at 0.3 h was significantly less than the percent dose fed at 4, 5 and 7 h (*, $P<0.05$, Student’s t-test).
Figure 4.
Intactness of ingested $^{125}$I$sCD14$ in the blood of 10 d old rat pups post size separation (< or >30 kDa) visualized by phosphor-imaging (A) and silver staining (B). Intact and non-ingested $^{125}$I$sCD14$ was used as a positive control. Figure is representative of n=4.
Figure 5.
The presence of rat-sCD14 in rat milk, 10 days postpartum, as detected by immunoblotting. Lanes: 1, molecular weight marker; 2, rat milk; 3, human recombinant sCD14 (100 ng, positive control).