

CD14: A Soluble Pattern Recognition Receptor in Milk

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Abstract An innate immune system capable of distinguishing among self, non-self, and danger is a prerequisite for health. Upon antigenic challenge, pattern recognition receptors (PRRs), such as the Toll-like receptor (TLR) family of proteins, enable this system to recognize and interact with a number of microbial components and endogenous host proteins. In the healthy host, such interactions culminate in tolerance to self-antigen, dietary antigen, and commensal microorganisms but in protection against pathogenic attack. This duality implies tightly regulated control mechanisms that are not expected of the inexperienced neonatal immune system. Indeed, the increased susceptibility of newborn infants to infection and to certain allergens suggests that the capacity to handle certain antigenic challenges is not inherent. The observation that breast-fed infants experience a lower incidence of infections, inflammation, and allergies than formula-fed infants suggests that exogenous factors in milk may play a regulatory role.

There is increasing evidence to suggest that upon exposure to antigen, breast milk educates the neonatal immune system in the decision-making processes underlying the immune response to microbes. Breast milk contains a multitude of factors such as immunoglobulins, glycoproteins, glycolipids, and antimicrobial peptides that, qualitatively or quantitatively, may modulate how neonatal cells perceive and respond to microbial components. The specific role of several of these factors is highlighted in other chapters in this book. However, an emerging concept is that breast milk influences the neonatal immune system's perception of "danger." Here we discuss how CD14, a soluble PRR in milk, may contribute to this education.

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The Discovery of CD14

CD14, first described in 1990 as a receptor for the bacterial endotoxin lipopolysaccharide (LPS) (Wright et al., 1990), is the first documented PRR (Pugin et al., 1994). Originally characterized as a monocyte/macrophage differentiation antigen, it is constitutively expressed by a variety of other cell types, including polymorphonuclear neutrophils, chondrocytes (Goyert & Ferrero, 1987; Matsuura et al., 1994; Tobias & Ulevitch, 1993), B cells (Schumann et al., 1994), dendritic cells (Verhasselt et al., 1997), gingival fibroblasts (Watanabe et al., 1996; Sugawara et al., 1998), keratinocytes (Song et al., 2002), hepatocytes (Liu et al., 1998), tracheal epithelial cells (Diamond et al., 1996), and human intestinal epithelial cell lines (Funda et al., 2001). However, a soluble form of CD14 (sCD14) also exists. First discovered in cell culture supernatants and in normal serum that blocked monocyte staining with anti-CD14 monoclonal antibodies (Maliszewski et al., 1985), sCD14 has been described in substantial concentrations in serum (Landmann et al., 1996; Bazil et al., 1986), cerebrospinal fluid (Cauwels et al., 1999), urine (Bazil et al., 1989), seminal plasma (Harris et al., 2001), saliva (Uehara et al., 2003), tears (Blais et al., 2005), and breast milk (Labeta et al., 2000; Vidal et al., 2001).

Molecular Characteristics of CD14

Genomic DNA encoding human CD14 was cloned in 1988 (Ferrero & Goyert, 1988). The CD14 gene has been mapped to region 5q 23-31 of chromosome 5, a region that codes for growth factors and receptors (Goyert et al., 1988). It consists of only two exons, starting with an ATG sequence directly followed by an 88-bp intron. A single mRNA species is translated.

During processing in the endoplasmic reticulum, a 19 amino acid-signal sequence is removed (Haziot et al., 1988) to yield the mature human membrane CD14 (mCD14) protein, which is composed of 356 amino acids with multiple leucine-rich repeats (Ferrero et al., 1990) and has sites for N-linked and O-linked glycosylation (Stelter et al., 1996). The sequence ends with a 22 amino acid-hydrophobic domain that lacks the characteristic basic residues of a stop transfer domain. CD14 is attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor that is added to the C terminus in the endoplasmic reticulum (Haziot et al., 1988; Simmons et al., 1989). CD14 has a pI of 4.5–5.1 (Nasu et al., 1991) and a carbohydrate content that accounts for about 20% of the total molecular mass of the mature 53-kDa glycoprotein (Bazil et al., 1986).

The primary sequence of human CD14 is highly homologous (61–73%) to the deduced amino acid sequence of its mouse (Setoguchi et al., 1989), rat (Takai et al., 1997), rabbit (Tobias et al., 1992), and bovine (Ikeda et al., 1997) counterparts. The bovine CD14 cDNA, isolated from a genomic library

(Ikeda et al., 1997), encodes a protein of 373 amino acids whose coding sequence is separated by a 90-nucleotide intron.

Soluble Forms of CD14

In normal human plasma, sCD14 occurs at concentrations of about 3 $\mu\text{g/mL}$ (Bazil et al., 1989; Grunwald et al., 1992; Durieux et al., 1994), but in human milk, its concentrations are ~ 10 -fold higher (Table 1) (Labeta et al., 2000; Vidal et al., 2001). Of note, sCD14 has also been detected in bovine colostrum (Labeta et al., 2000; Filipp et al., 2001) and milk (Filipp et al., 2001). Quantification of sCD14 in bovine milk has been difficult due to the lack of a specific assay. Nevertheless, it is estimated to be present in quantities that are slightly inferior to those in human milk (Lee et al., 2003a; Vangroenweghe et al., 2004).

The origin of sCD14 is uncertain. There are many potential sources for sCD14, including cleavage of the receptor from monocytes by proteases or phospholipases (Bazil et al., 1989; Bazil & Strominger, 1991), and direct secretion of full-length molecules that have bypassed the GPI-linking mechanism (Labeta et al., 1993; Durieux et al., 1994; Bufler et al., 1995). Cells treated with phosphoinositol-phospholipase C release CD14, which migrates in SDS-PAGE as a doublet in the 50-kDa range. In contrast, CD14 released via the action of cellular proteases migrates as a single molecular-weight moiety in SDS-PAGE (Bazil & Strominger, 1991).

At least two soluble forms of CD14 are constitutively generated in serum (Bazil et al., 1989; Durieux et al., 1994; Stelter et al., 1996), one form is a ~ 50 -kDa molecule that is released after shedding of the cell surface form (Bazil et al., 1986; Haziot et al., 1988, 1993b). A second form, with higher molecular weight (~ 56 kDa), is released from cells before addition of the GPI anchor (Labeta et al., 1993; Landmann et al., 1995; Bufler et al., 1995). The sCD14 found in serum and urine appears to be a mixture of the two forms. More recently, a new isoform of sCD14, named sCD14 subtype, has been identified in the serum of patients with sepsis (Yaegashi et al., 2005).

The sCD14 present in human milk during most of the lactating period is a single molecular form of ~ 48 kDa (Labeta et al., 2000; Vidal et al., 2001). This is identical to that produced by differentiated mammary epithelial cells *in vitro* (Labeta et al., 2000; Vidal et al., 2001) and suggests that the mammary gland

Table 1 Levels of sCD14 in Human Breast Milk During Lactation

	Days Postpartum		
	≤ 6 days	≥ 8 days	0–71 days
sCD14	20.10 ± 8.74	13.09 ± 4.31	14.84 ± 6.39
($\mu\text{g/mL}$)	($n = 10$)	($n = 30$)	($n = 40$)

Data represent mean $\pm$ standard deviation of samples tested by enzyme-linked immunosorbent assay.

epithelium is the main source of milk sCD14. Interestingly, the early milk samples have a complex sCD14 pattern with three polypeptide bands (~48, 50, and 56 kDa). It is not known whether these different forms reflect distinct glycosylation patterns and/or different sizes of the core proteins. However, since milk macrophages express the typical serum sCD14 isoforms (i.e., 50 and 56 kDa), their production of sCD14 may contribute to the total pool of sCD14 within the first week postpartum. It is also possible that there is leakage of serum sCD14 into the milk via mammary gland alveolar cell tight junctions or by transient active transport.

Regulation of sCD14 Expression

In vitro studies show that while treatment of blood monocytes with LPS and TNF- α causes an increase in serum sCD14 levels, exposure to IFN- γ and IL-4 decreases the levels (Schutt et al., 1992; Landmann et al., 1992). Little is known about the regulation of sCD14 expression *in vivo*. However, increased circulating sCD14 levels correlate with infectious, autoimmune, and inflammatory diseases (Kruger et al., 1991; Nockher et al., 1994; Yu et al., 1998). In addition, it has been demonstrated that sCD14 can be considered as a type 2 acute-phase protein (APP) (Bas et al., 2004). Liver levels of CD14 mRNA increase in IL-6/- mice injected with turpentine, an experimental model of acute-phase response (Bas et al., 2004). Furthermore, serum sCD14 levels in patients with various arthropathies correlate with those of C-reactive protein, a classical APP, and with IL-6, a cytokine known to regulate the synthesis of APP in the liver. Serum levels of sCD14 also correlate with disease activity in rheumatoid arthritis and reactive arthritis patients.

Interestingly, human milk sCD14 levels correlate with those of specific fatty acids (Dunstan et al., 2004; Laitinen et al., 2006) and are influenced by fish oil supplementation in the maternal diet (Dunstan et al., 2004). In bovine milk, sCD14 levels are elevated during mastitis or following intramammary challenge with LPs or *Escherichia coli* (Bannerman et al., 2003; Lee et al., 2003a, b; Vangroenweghe et al., 2004) and may be due to infiltration of neutrophils to the inflamed mammary gland.

Although highly conserved across a wide range of species, genes involved in innate immunity demonstrate considerable interethnic variability predominantly as single-nucleotide polymorphisms (SNPs). It has been recently demonstrated that genetic variation in the promoter region of the cDNA encoding CD14 affects sCD14 levels (LeVan et al., 2006; Baldini et al., 1999). No correlation has been observed between CD14 promoter polymorphisms at positions -4190, -2838, -1720, and -260 and the levels of sCD14 at birth. However, an association between genotypes and sCD14 is evident at three months of age, and longitudinal analyses suggest that CD14 polymorphisms

modulate sCD14 levels through the first year of life in healthy infants (LeVan et al., 2006).

Interestingly, it has been reported that sCD14 levels in plasma and milk are differentially regulated by the same genetic variants (Guerra et al., 2004a). More specifically, plasma and milk sCD14 levels differ significantly both by CD14/-1619 and CD14/-550 genotypes and by haplotypes. Moreover, the CD14/-550T allele and the corresponding ATC haplotype are associated with high levels of sCD14 in milk but low levels of sCD14 in plasma. Taken together, these suggest the existence of cell-specific regulation of CD14 gene expression in the two compartments (Guerra et al., 2004a).

Role of CD14 in the Host Response to Bacterial Ligands

Host–Microbe Interactions

Our understanding of microbiota–host immune system interactions has made great progress in recent years, and there is increasing evidence to suggest that such interactions benefit both the bacteria and the host. Intestinal epithelial cells (IEC) are at the interface between the luminal environment and the mucosal immune system. For immune homeostasis and effective immune defense, these cells, together with sentinel dendritic cells, sense the bacterial load in the intestinal lumen and determine the outcome of the primary, innate response.

In healthy individuals, transient local immune responses but not systemic responses are initiated against the intestinal flora (Macpherson & Harris, 2004; Haller et al., 2000), while active and more aggressive responses are generated against the “danger signals” from pathogens (Matzinger, 2002). It is now clear that the responses to commensals are essential for the development and maturation of the intestinal immune system (Cebra, 1999; Macpherson & Harris, 2004), the integrity of the intestinal epithelium, the maintenance of immune homeostasis, as well as for the production of antimicrobial peptides and tissue repair (Rakoff-Nahoum et al., 2005). Indeed, the hygiene hypothesis suggests that in early life, a modified interaction between microbial antigens and the innate immune system underlies the increased incidence of allergic and autoimmune diseases in developed countries (Bloomfield et al., 2006). The original hypothesis arose from epidemiological studies that reported an inverse association between family size and the development of atopy (Strachan, 1989). Later studies have found a similar relationship using other measures of microbial exposure such as farm living, bed sharing, and attending nursery school as well as more direct forms of exposure such as infection, exposure to endotoxins, food-borne microbes, or the gut microbiota (Bloomfield et al., 2006). In this context, it is interesting that lower circulating levels of sCD14 levels in children are associated with the development of atopy (Zdolsek & Jenmalm, 2004) and

wheezing (Guerra et al., 2004b) and that blood cells from farmers' children have higher amounts of CD14 mRNA than those from nonfarmers' children (Lauener et al., 2002).

CD14 as a PRR

Wright et al. (1990) were the first to identify the monocyte differentiation antigen CD14 as a key monocyte receptor for bacterial LPS. The binding stoichiometry of LPS to CD14 has been reported to be one to one (Kitchens & Munford, 1995), with a K_d value of 27 nM (Kirkland et al., 1993). However, since LPS tends to aggregate in solution, stoichiometric data are conflicting. It is through the formation of large ternary complexes consisting of LPS, CD14, and lipopolysaccharide binding protein (LBP) (Gegner et al., 1995) that monocytes detect the presence of LPS (Thomas et al., 2002). Cellular activation begins when the acute-phase protein LBP binds to LPS and catalyzes its binding to CD14 on the cell surface (Tobias et al., 1986; Schumann et al., 1990; Wright et al., 1990; Mathison et al., 1992; Martin et al., 1992; Hailman et al., 1994). Binding and phagocytosis of whole Gram-negative bacteria is also mediated by membrane CD14 in an LBP-dependent manner (Grunwald et al., 1996). It is noteworthy that serum from LBP knockout mice is unable to mediate LPS-induced oxidative burst responses in mouse peritoneal exudate cells (Jack et al., 1997).

Since CD14 lacks a cytoplasmic domain, it cannot signal the presence of LPS. Rather, the transmembrane TLR family of proteins, which discriminate conserved motifs present in pathogens and commensals, transduce intracellular signals (Aderem & Ulevitch, 2000). To date, 12 mouse and 10 human TLRs capable of recognizing single or multiple motifs from bacteria, viruses, and fungi have been identified (Kaisho & Akira, 2006). Of these, the most studied TLR for bacterial recognition are TLR2, which recognizes the lipoteichoic acid (LTA) and peptidoglycan (PGN) from Gram-positive bacteria; TLR4 and TLR5, which bind, respectively, to the lipopolysaccharide (LPS) and the flagellin from Gram-negative bacteria; and TLR9, the receptor for unmethylated CpG DNA. The discoveries that TLR4 mediates LPS-induced signal transduction (Chow et al., 1999; Takeuchi et al., 1999) and that for some bacterial ligands, TLR cooperate with other soluble and cell-surface proteins led to CD14's reclassification as a co-receptor for LPS.

It is now known that for intracellular signal transduction, LPS is transferred from the CD14-LBP complex to TLR4 that is bound to myeloid differentiation protein (MD)-2 on the cell surface (da Silva et al., 2001). TLR belong to the TLR/IL-1R superfamily whose members have a Toll/IL-1 receptor (TIR) domain (O'Neill, 2002). In response to ligand binding, this cytoplasmic TIR domain then sequentially recruits a series of adapter molecules including myeloid differentiation marker-88 (MyD88), IL-1 receptor-

associated kinase (IRAK), and tumor necrosis factor receptor-associated factor (TRAF)-6. Ultimately, this recruitment activates nuclear factor (NF)- κ B and mitogen-activated proteins (MAP) kinases (O'Neill et al., 2003) and induces the production of pro-inflammatory cytokines such as IL-8, TNF- α , and IL-1 (Dentener et al., 1993), as well as anti-inflammatory cytokines (e.g., IL-10, TGF- β).

LPS is not the only bacterial ligand recognized by CD14. In 1994, Pugin et al. (1994) also identified CD14 as a PRR for PGN, the major cell wall component of Gram-positive bacteria and for lipoarabinomannan, a glycolipid from *Mycobacterium tuberculosis*. Indeed, it is now known that several other microbial ligands from bacteria, yeast, and spirochetes are able to interact with CD14 (Table 2) (Heumann et al., 1998). In addition, CD14 may function as a receptor for several endogenous human proteins such as heat shock protein 60 (Kol et al., 2000), ceramide, anionic phospholipids, modified lipoproteins, and opsonized particles (Schmitz & Orso, 2002).

Like that of LPS, cellular activation by the LTA and PGN of Gram-positive bacteria also involves a receptor complex comprising CD14, but instead of TLR4, signaling is transduced by TLR-2 (Schwandner et al., 1999; Schroder et al., 2003). Although both TLR4 and TLR2 ligands activate NF- κ B via MyD88, IRAK, and TRAF-6 (O'Neill et al., 2003), they elicit different biological responses. For example, TLR4 agonists promote dendritic cell production of the Th1-inducing

Table 2 Interactions of CD14 with Ligands Derived from Different Microbial Sources

Microbial Sources	Ligands
Gram-negative bacteria	LPS from <i>E. coli</i> ^a
	Whole Gram-negative <i>E. coli</i> ^b
	Polymannuronic acid from <i>Pseudomonas</i> ^c
Gram-positive bacteria	Lipoarabinomannan from <i>Mycobacteria tuberculosis</i> ^d
	Cell wall constituents from <i>Bacillus subtilis</i> ^e and <i>Staphylococcus aureus</i> ^f
	Soluble peptidoglycan from <i>Staphylococcus aureus</i> ^g
	Rhamnose glucose polymers of <i>Streptococcus mutans</i> ^h
	Lipotechoic acid (LTA) from <i>Staphylococcus aureus</i> and <i>Streptococcus pyrogenes</i> ⁱ
	Lipoproteins and lipopeptides from <i>Treponema palladium</i> and <i>Borrelia burgdorferi</i> ^j
Yeast	Peptide derived from the WI-1 protein of <i>Blastomyces dermatidis</i> ^k
Spirochetes	Outer surface protein (Osp) from <i>Borrelia burgdorferi</i> ^l

Sources: ^aGallay et al. (1993); ^bJack et al. (1995); ^cEspevik et al. (1993); ^dPugin et al. (1994), Savedra et al. (1996), Zhang et al. (1993); ^ePugin et al. (1994); ^fKusunoki et al. (1995), Kusunoki and Wright (1996); ^gWeidemann et al. (1994), Gupta et al. (1996), Weidemann et al. (1997), Dziarski et al. (1998); ^hSoell et al. (1995); ⁱCleveland et al. (1996), Hattor et al. (1997); ^jSellati et al. (1998), Wooten et al. (1998); ^kNewman et al. (1995); ^lWooten et al. (1998)

cytokine IL-12p70 and the chemokine, interferon-inducible protein (IP)-10. In contrast, TLR2 agonists fail to stimulate the production of these proteins but rather induce the production of the IL-12 inhibitory p40 homodimer that favors Th2 development (Re & Strominger, 2001).

Roles of sCD14

Like membrane CD14 (mCD14), sCD14 also forms a complex with LPS (Vita et al., 1997) that is capable of activating both CD14-positive cells and CD14-negative cells, such as epithelial, endothelial, and smooth muscle cells (Frey et al., 1992; Pugin et al., 1993; Haziot et al., 1993a; Arditi et al., 1993; Goldblum et al., 1994; Loppnow et al., 1995; Read et al., 1993). This complex can stimulate cells even in the absence of LBP (Hailman et al., 1994). However, the nature of the cellular response depends on the concentration of LPS, sCD14, and LBP present (Kitchens & Thompson, 2005). Indeed, the range of sCD14 concentrations found in normal and septic humans can significantly decrease monocyte responses to LPS. By competing with mCD14, the soluble form limits the amount of LPS binding to the cells (Jacque et al., 2006) and thereby inhibits LPS-induced cellular activation (Grunwald et al., 1993; Haziot et al., 1994; Schutt et al., 1992). However, in addition to binding bacterial motifs, sCD14 also binds phospholipids, such as phosphatidylinositol and phosphatidylethanolamine, and transfers them to high-density lipoproteins (Yu et al., 1997). It also mediates the influx of phospholipids into cells as well as their efflux out of cells and into plasma (Sugiyama & Wright, 2001). By shuttling LPS from mCD14 to plasma lipoproteins (Yu et al., 1997; Kitchens et al., 1999, 2001), sCD14 may retain LPS in the circulation and prevent LPS-mediated lethality (Jacque et al., 2006). The suggestion that LPS moves between the membrane and soluble forms of CD14 until an equilibrium is reached and is progressively removed from both forms when plasma lipoproteins are present (Kitchens & Thompson, 2005) may explain the apparent ambiguity in sCD14 function.

Relevance of sCD14 for Infant Health

The fetal intestine is sterile, and the events governing colonization and subsequent assembly of the intestinal microbiota remain elusive. At parturition, the immature intestinal immune system is immediately challenged by a massive bacterial insult in the birth canal and the external environment. In the early postnatal period, the composition of the microbiota constantly changes as a large number of organisms compete for an intestinal niche. However, some stability is achieved around weaning (Edwards & Parrett, 2002) and maintained throughout adulthood (Zoetendal et al., 1998). It is remarkable that the

inexperienced neonatal immune system accommodates all these changes in the absence of adverse immune responses and finally permits an estimated 10^{14} bacteria from at least 400 different species (Berg, 1996) to establish themselves in the intestinal lumen. Clearly, in the full-term, healthy infant, basic protective measures to ensure immune tolerance to commensals, even in the face of pathogenic attack, are already in place at birth. However, interaction with a high density of microbial antigens from a wide spectrum of species is a potentially hazardous process, particularly for preterm infants. Indeed, an inappropriate inflammatory response to intestinal microorganisms may contribute to the development of pathological conditions such as necrotizing enterocolitis (NEC) (Caplan & MacKendrick, 1993; Hoy et al., 1990). Furthermore, perturbations in the microbiota can lead to septicemia (van Saene et al., 2003) or allergic disease (Kirjavainen et al., 2002) in infants.

In the adult intestine, low expression of TLR2 and TLR4 (Otte et al., 2004; Abreu et al., 2001) and the absence of MD2 (Abreu et al., 2001) on IEC could explain, at least in part, the lack of response to the microbiota. On the other hand, fetal IEC express both TLR2 and TLR4 and are hyperresponsive to LPS (Claud et al., 2004; Fusunyan et al., 2001). Indeed, an animal model suggests that an interaction between intestinal bacteria and neonatal IEC expressing high levels of TLR4 underlies the development of NEC (Jilling et al., 2006). Thus, increased expression of TLR on fetal IEC could explain the increased susceptibility of preterm infants to NEC. Continuous activation of IEC in the immediate postnatal period may cause downregulated TLR expression on IEC (Abreu et al., 2001) and tolerance to endotoxins (Lotz et al., 2006). It is not known if soluble forms of CD14 influence the outcome of such activation. However, cellular activation by LPS can lead to shedding of CD14 in a soluble form and subsequent downregulation of inflammatory cytokine production (Sohn et al., 2007; Kitchens & Thompson, 2005).

Amniotic fluid contains sCD14, the concentration of which is increased with intrauterine infection and preterm labor (Espinoza et al., 2002). It is possible that sCD14 in the amniotic fluid still bathes mucosal surfaces during the birthing process and determines the extent of the initial microbial interaction with the associated lymphoid tissue. It is also possible that breast milk sCD14 continues this in the postnatal period. To date, few studies have examined the effect of sCD14 in amniotic fluid or breast milk on host–microbe interactions, the immune status of the infant, or the development of infection and disease. Nevertheless, the levels of sCD14 in amniotic fluid and breast milk are associated with subsequent development of atopy, eczema, and asthma (Jones et al., 2002; Rothenbacher et al., 2005).

Breastfeeding certainly influences the composition of intestinal microbiota (Falk et al., 1998) and protects against infection (Lonnerdal, 2003), bacterial translocation in neonatal animals, and septicemia and NEC in premature human infants (Steinwender et al., 1996; Ronnestad et al., 2005). It also reduces the risk of developing asthma, atopic dermatitis, eczema, and allergy (Kull et al., 2004; Lawrence, 2005) and pathological diseases later in life

(Jackson & Nazar, 2006). It is plausible that regulatory factors in breast milk educate the neonatal immune system to recognize and respond appropriately to bacterial components and that milk sCD14 may contribute to such an education. Certainly, human breast milk contains high levels of sCD14 (Labeta et al., 2000; Filipp et al., 2001), which may participate in the transient activation of the innate immune response to bacterial components and its subsequent downregulation. For example, milk sCD14 mediates the LPS-induced production of the pro-inflammatory cytokines IL-8 and TNF- α and of the chemokine ENA-78 by IEC (Labeta et al., 2000) as well as the production of IL-8 by monocytes and dendritic cells (Labeta et al., 2000; Lebouder et al., 2006). An excessive, unresolved inflammatory response may be avoided by the limited amount of LBP in milk (Vidal et al., 2001) and/or by the presence of other milk proteins such as lactoferrin, which has been shown to inhibit the LPS-induced production of pro-inflammatory mediators via NF- κ B (Haversen et al., 2002) as well as the sCD14-LPS-induced expression of IL-8 and adhesion molecules by endothelial cells (Elass et al., 2002; Baveye et al., 2000). Interestingly, milk sCD14 does not mediate the production of pro-inflammatory cytokines by IEC exposed to Gram-positive bacteria or their LTA *in vitro* (Vidal et al., 2002). This differential response may be due to the expression of TLR4 and absence of TLR2 on the cell line used. However, LTA inhibits the LPS-milk sCD14-induced response, most probably by competitive binding to the sCD14 molecule (Vidal et al., 2002). It is also noteworthy that sCD14 mediates LPS-induced expression of IL-6, IL-8, IL-12, and co-stimulatory molecules by dendritic cells, which, like IEC, do not express mCD14 (Verhasselt et al., 1997).

Direct immunoregulatory effects of sCD14 on activated T and B cells have also been reported. More specifically, sCD14 inhibits the proliferation of activated T cells and their production of the Th1 cytokines IL-2 and IFN- γ and the Th2 cytokine IL-4 (Rey Nores et al., 1999). It also induces a progressive accumulation of I κ B α , an inhibitor of NF- κ B. In B cells, sCD14 interferes with the CD40 signaling pathway and the production of IgE (Arias et al., 2000) and, when administered to neonatal mice, bovine milk-derived sCD14 induces immunoglobulin secretion (Filipp et al., 2001). A correlation between sCD14 concentrations in colostrum and the numbers of IgA and IgM secreting cells in human neonates lends further support to this observation (Rinne et al., 2005).

It is tempting to speculate that sCD14 in milk instigates a beneficial, innate immune response to specific microbes in sentinel IEC and dendritic cells that lack mCD14 and thwarts an exaggerated response to microbial antigens through the production of protective immunoglobulins and the regulation of effector T cells. The observation that breast milk sCD14 survives intact in conditions that mimic the upper digestive tract but is digested by pancreatin (Blais et al., 2006) suggests that such innate responses are initiated in an environment with low bacterial density but are avoided in the densely populated distal intestine.

Relevance of sCD14 to Pathological Diseases

The expression and activation of TLRs in the gastrointestinal tract must be tightly regulated to prevent unrelenting inflammation in the face of microbial exposure. Ideally, molecules coordinating TLR effects should limit but not completely eliminate microbial interaction with the mucosal immune system. The previous section suggests that sCD14 possesses this quality and contributes to immune homeostasis in the healthy individual. However, such an attribute may secure immune defense and prevent a dysregulated inflammatory response during infection or aberrant TLR signaling. Admittedly, increased levels of sCD14 are associated with the severity of infection in Gram-positive sepsis (Burgmann et al., 1996) and, notably, with a high mortality in Gram-negative septic shock (Landmann et al., 1995), but to date, there is no clear indication whether these relationships are the cause or effect. Nevertheless, some *in vivo* animal studies suggest that increased levels of sCD14 can counteract the detrimental effects of LPS. For example, levels of sCD14 increase in milk following mammary gland infection or the injection of LPS (Lee et al., 2003a; Vangroenweghe et al., 2004), and the administration of recombinant sCD14 has been shown to reduce the infection in mice (Lee et al., 2003c) and cows (Lee et al., 2003b; Nemchinov et al., 2006). Recombinant sCD14 also protects against mortality in mice treated with LPS (Stelter et al., 1998; Haziot et al., 1995). Furthermore, in transgenic mice expressing different copy numbers of the human CD14 transgene on a murine CD14^{-/-} background, mice with high levels of human CD14 retain LPS in the circulation and prevent its delivery to tissues and organs (Jacque et al., 2006). In so doing, these mice are hyporesponsive to LPS and survive a lethal dose (Jacque et al., 2006).

CD14 is an APP, and several clinical studies have reported increased serum levels of sCD14 in a range of inflammatory conditions (Bas et al., 2004). Higher serum levels are associated with several insulin-resistance-related phenotypes (Fernandez-Real et al., 2003), systemic lupus erythematosus (Egerer et al., 2000), atopic dermatitis (Wuthrich et al., 1992), systemic inflammatory response syndrome (Stoiser et al., 1998), angina (Zalai et al., 2001), preterm labor even in the absence of infection (Gardella et al., 2001), multiple organ failure (Endo et al., 1994), rheumatoid arthritis (Horneff et al., 1993; Yu et al., 1998), multiple sclerosis (Lutterotti et al., 2006), Kawasaki disease (Takeshita et al., 2000), and Gaucher's disease (Hollak et al., 1997). These associations may reflect CD14's capacity to bind to nonmicrobial factors such as monosodium urate crystals (Scott et al., 2006), host heat shock proteins (Kol et al., 2000), integrins (Humphries & Humphries, 2007), surfactant proteins (Sano et al., 2000), atherogenic lipids, and lipoproteins (Schmitz & Orso, 2002), but they also suggest that sCD14 may modulate host immune responses to other "danger" signals besides those of microbial origin.

Conclusion

An emerging concept is that breast milk influences the neonatal immune system's perception of "danger." To do so, soluble PRRs in milk, such as sCD14, may actually facilitate the intestinal response to specific microbial motifs by activating intracellular signaling pathways such as that of NF- κ B, a process necessary for maturation of immune tissues. There is evidence that sCD14 mediates both pro- and anti-inflammatory responses depending on the type and location of the responder cell and the nature and dose of the stimulus. It is tempting to speculate that the presence of this versatile molecule in breast milk instructs the neonatal immune system to recognize and respond appropriately to self, non-self, and different forms of danger. To date, little work has specifically addressed the biological activity of milk sCD14 or the function of sCD14 administered orally. Nevertheless, the possibility of developing sCD14-containing products using animal milk holds much promise, not only for infant nutrition but also for clinical application.

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