Intestinal Macrophages Lack CD14 and CD89 and Consequently Are Down-Regulated for LPS- and IgA-Mediated Activities

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The intestinal mucosa normally displays minimal inflammation despite the close proximity between mucosal macrophages and luminal bacteria. Macrophages interact with bacteria and their products through CD14, a surface receptor involved in the response to LPS, and CD89, the receptor for IgA (FcαR). Here we show that resident macrophages isolated from normal human intestine lack CD14 and CD89. The absence of CD14 and CD89 was not due to the isolation procedure or mucosal cell products, but was evident at the transcriptional level, as the macrophages expressed neither CD14- nor CD89-specific mRNAs, but did express Toll-like receptor 2 and 4 transcripts. Consistent with their CD14- phenotype, lamina propria macrophages displayed markedly reduced LPS-induced cytokine production and LPS-enhanced phagocytosis. In addition, IgA-enhanced phagocytosis was sharply reduced in lamina propria macrophages. Thus, the absence of CD14 and CD89 on resident intestinal macrophages, due to down-regulated gene transcription, causes down-modulated LPS- and IgA-mediated functions and probably contributes to the low level of inflammation in normal human intestinal mucosa. 

The intestinal mucosa is the largest surface to interface with the external environment (1) and the largest reservoir of macrophages (2) in the body. Strategically positioned in the subepithelial lamina propria, intestinal macrophages are the first phagocytic cells of the innate immune system to interact with micro-organisms and microbial products that have breached the epithelium. Such interactions are thought to involve CD14, a GPI-linked glycoprotein that acts as a high affinity receptor for complexes of LPS and LPS-binding protein (3), and CD89, a transmembrane glycoprotein receptor for monomeric and polymeric IgA1 and IgA2 (FcαR) (4). Because CD14 and CD89 are present on blood monocytes, and blood monocytes are thought to populate the mucosa (5,6), lamina propria macrophages have been assumed to express these receptors. However, we (7) have shown that intestinal macrophages lack CD14, and others (6,8) have shown that colonic macrophages express low levels of CD14. The effect of down-regulated CD14 expression on lamina propria macrophage function, which has not been previously investigated, has important implications for understanding the pathophysiology of the low level of mucosal inflammation in an environment rich in proinflammatory signals. Here we extend our previous findings by showing that purified human intestinal macrophages are devoid of surface CD14 as well as CD89 due to down-regulated CD14 and CD89 gene transcription, although they did express Toll-like receptor 2 (TLR2) and TLR4, receptors that transduce the LPS-stimulated signal (9). The absence of CD14 and CD89 markedly down-modulates LPS- and IgA-induced proinflammatory activities by resident intestinal macrophages.

Materials and Methods

Lamina propria macrophages and blood monocyte

Lamina propria macrophages were isolated by neutral protease digestion of intestinal tissue sections from individual donors and then purified by counterflow centrifugal elutriation, as described previously in detail (7,10,11). Briefly, sections of normal human jejunum obtained from healthy subjects undergoing gastrojejunostomy for morbid obesity were rinsed in Ca2+- and Mg2+-free PBS and washed in HBSS containing DTT (200 μg/ml) to remove residual mucus and then in HBSS containing 0.2 M EDTA plus 10 mM 2-ME to remove the epithelium. Sections were then minced and treated with the neutral protease dispase (75 μg/ml; grade I; sp. act. 6 U/mg with Limulus amebocyte lysate assay; Roche, Indianapolis, IN) to remove residual mucus and then in HBSS containing 0.2 M EDTA plus 10 mM 2-ME to remove the epithelium. Sections were then minced and treated with the neutral protease dispase (75 μg/ml; grade I; sp. act. 6 U/mg with Limulus amebocyte lysate assay; Roche, Indianapolis, IN) to remove residual mucus and then in HBSS containing 0.2 M EDTA plus 10 mM 2-ME to remove the epithelium. Sections were then minced and treated with the neutral protease dispase (75 μg/ml; grade I; sp. act. 6 U/mg with Limulus amebocyte lysate assay; Roche, Indianapolis, IN) to remove residual mucus and then in HBSS containing 0.2 M EDTA plus 10 mM 2-ME to remove the epithelium. Sections were then minced and treated with the neutral protease dispase (75 μg/ml; grade I; sp. act. 6 U/mg with Limulus amebocyte lysate assay; Roche, Indianapolis, IN) to remove residual mucus and then in HBSS containing 0.2 M EDTA plus 10 mM 2-ME to remove the epithelium.

Flow cytometry and Abs

Lamina propria macrophages, which were cultured for 2 days before study, and blood monocytes were preincubated in PBS with 10% normal human...
Phagocytic activity for FITC-labeled microspheres coated with either LPS (17, 18) or IgA (19–21) was assessed by FACS. Briefly, 1 μl latex microspheres (Polysciences, Warrington, PA) were coated with Porphyromonas gingivalis LPS (10 μg/10^6 microspheres/100 μl) or human serum IgA1 (3 μg/10^6 microspheres/100 μl) and subsequently blocked with 1% BSA (Sigma). The LPS was purified from P. gingivalis 33277 following hot phenol extraction, and the IgA1 was purified from human AB serum by lectin affinity chromatography. Control microspheres were coated with BSA only. Microspheres were added to lamina propria macrophages or blood monocytes in RPMI 1640 with 10% FBS (Life Technologies, Grand Island, NY) at a ratio of 10 microspheres/cell for 3 h at 37°C in 5% CO₂. After washing to remove free microspheres, the macrophages and monocytes were fixed with 1% paraformaldehyde and analyzed by FACS. Previous studies showed that a shift in fluorescence intensity reflected bead uptake and not adherence (7).

**Results**

**Lamina propria macrophages do not express surface CD14 or CD89**

The cells isolated from the lamina propria displayed macrophage morphology with pseudopod projections from the cell membrane; an eccentric, often concave, nucleus; phagocytic vacuoles; and secondary lysosomes (Fig. 1); they contained nonspecific esterase and displayed phagocytic activity (7) (see below). Cell purity analysis showed that the cells expressed high levels of surface HLADR and CD13, typical of macrophages (7) (see below), but not the dendritic cell markers CD11b, CD11c, CD21, CD34, CD83, or CD123, and treatment of the cells with optimal concentrations of GM-CSF, TNF-α, and IL-4 did not induce CD83 or dendritic cell morphology. The cells also did not express the lymphocyte markers CD3 and CD103.

Intestinal lamina propria macrophages were analyzed in parallel with purified blood monocytes for surface CD14, CD89, CD13, and HLADR. Greater than 98% of the lamina propria macrophages and blood monocytes expressed CD13 and HLADR (Fig. 2, lower panels). However, lamina propria macrophages invariably displayed no surface CD14 or CD89 (Fig. 2, right upper panel), whereas blood monocytes expressed both CD14 and CD89 (Fig. 2, left upper panel). The absence of CD14 on the lamina propria

**FIGURE 1.** Ultrastructural features and distribution of intestinal lamina propria macrophages. Isolated intestinal lamina propria macrophages display pseudopods, phagocytic vacuoles, secondary lysosomes, and a large eccentric nucleus (×10,000). Inset. Macrophages stained with HAM56 are abundantly distributed throughout the lamina propria in this section of normal jejunum (×32).
macrophages was confirmed with seven additional anti-CD14 mAbs (MO2, MY4, FMC17, TUK4, YMEM18, UCHM-1, FMC-32/2D-15C), and the absence of CD89 was confirmed with three additional anti-CD89 mAbs (A62, A77, MY43). One explanation for the absence of CD14 and CD89 on lamina propria macrophages is that the receptors are neutral protease sensitive. However, blood monocytes treated with dispase expressed CD14 (LeuM3) and CD89 (A59; Fig. 2) and showed no reduction in receptor expression using the above additional CD14 and CD89 mAbs (data not shown), indicating that the absence of CD14 and CD89 on lamina propria macrophages was not due to the isolation procedure.

Alternatively, the absence of CD14 and CD89 on lamina propria macrophages could have occurred in vivo in response to locally produced cytokines, such as IL-4 and IL-13, which down-regulate CD14 expression (22, 23), or IFN-γ and TGF-β1, which down-regulate CD89 (24, 25). To address this possibility, elutriated blood monocytes were analyzed for CD14 and CD89 expression before and after a 24-h exposure to mucosal cell-conditioned medium (dilution 1/2) prepared from 24-h cultures of purified jejunal epithelial cells (1 × 10⁷ cells/ml), lamina propria mononuclear cells (1 × 10⁷ cells/ml), and the lamina propria stroma after removal of the mononuclear cells (wet weight, 1 g/ml). Monocytes exposed to these mucosal cell-conditioned medium displayed no reduction in the percentage of cells that expressed either CD14 or CD89 (Table 1) or receptor density (data not shown). In addition, lamina propria macrophages cultured for 56 days in the absence of mucosal factors with and without LPS did not express CD14 or CD89. Together, these findings suggest that products present in the local microenvironment did not remove CD14 and CD89 from the macrophage cell surface.

**Absence of CD14⁺ and CD89⁺ cells in the lamina propria of jejunal tissue sections**

To confirm that the absence of surface CD14 and CD89 on the lamina propria macrophages was not due to the isolation procedure, we performed immunohistochemical analysis of jejunal tissue sections, as well as spleen and lung sections from the same donor, for the presence of CD14⁺ and CD89⁺ macrophages. Jejunal tissue contained numerous lamina propria HAMS56⁺ macrophages (Fig. 3A, inset) that expressed no CD14 (Fig. 3A) or CD89 (Fig. 3B). In sharp contrast, spleen tissue contained many CD14⁺ and CD89⁺ macrophages (Fig. 3, C and D), as did lung tissue (Fig. 3, E and F). Thus, the absence of CD14 and CD89 on isolated jejunal macrophages accurately reflected the absence of these receptors on lamina propria macrophages in situ and was not the consequence of the isolation procedure.

**Absence of CD14 and CD89 mRNA expression in lamina propria macrophages**

Because neither the isolation procedure nor exposure to the products of other mucosal cells appeared to account for the CD14⁻ CD89⁻ phenotype of lamina propria macrophages, we evaluated the cells for CD14 and CD89 mRNA expression. Lamina propria macrophages from four donors contained no detectable transcripts for either receptor, whereas blood monocytes from two donors expressed mRNA for both receptors (Fig. 4). The absence of CD14 and CD89 mRNA transcripts in lamina propria macrophages suggested that down-regulated gene expression accounted for the unique CD14⁻ CD89⁻ phenotype of resident intestinal macrophages.

**Lamina propria macrophages express mRNA for TLR2 and TLR4**

Although CD14 binds LPS, the signal induced by LPS is mediated by TLRs, recently identified transmembrane proteins that contain an intracellular signaling domain (26, 27). Therefore, to determine whether the absence of CD14 mRNA in lamina propria macrophages involved these important receptors as well, we evaluated lamina propria macrophages for the expression of TLR2 and TLR4 mRNA. As shown in Fig. 5, lamina propria macrophages from three separate donors expressed message for both TLR2 and TLR4 (Fig. 5). In addition, the four macrophage RNA preparations that did not contain CD14 or CD89 mRNA shown in Fig. 4 were also analyzed by RT-PCR for TLR2 and 4 mRNA, and despite the absence of CD14 and CD89 transcripts, TLR2- and TLR4-specific mRNA was present (data not shown). Thus, the absence of message for CD14 did not extend to transcripts of the TLR2 and 4 components of the LPS receptor complex.

**Reduced LPS- and IgA-stimulated activities by lamina propria macrophages**

Because macrophages play a fundamental role in orchestrating inflammatory responses to enteric bacteria and their products, we next evaluated lamina propria macrophages for LPS-stimulated cytokine (IL-8) production and LPS- and IgA-enhanced phagocytosis. In the absence of serum, LPS-stimulated lamina propria macrophages produced 100-fold less IL-8 than blood monocytes (Fig.
Moreover, LPS-stimulated production of IL-8 by the macrophages was not enhanced in the presence of serum as a source of LPS-binding protein, in contrast to LPS-stimulated IL-8 production by blood monocytes, which increased severalfold in the presence of serum. In addition, the macrophages produced markedly less IL-1, IL-6, TNF-α, and TGF-β than blood monocytes in response to LPS, and production of these cytokines also was not enhanced in the presence of serum (data not shown).

To further explore whether the absence of CD14 could account for the inability of lamina propria macrophages to release cytokines in response to LPS, we preincubated blood monocytes with increasing doses of anti-CD14 (MEM-18) Ab and quantitated the amount of IL-8 released after subsequent LPS stimulation. As

**FIGURE 3.** Immunohistochemical analysis of jejunal, spleen, and lung tissue for CD14⁺ and CD89⁺ cells. Serial sections of jejunum (A and B, ×160), spleen (C and D, ×160), and lung (E and F, ×400) from the same otherwise healthy organ transplantation donor were analyzed in parallel by immunohistochemistry for CD14⁺ (A, C, and E) and CD89⁺ (B, D, and F) cells as described in Materials and Methods. Jejunum was also stained for HAM56⁺ macrophages (A, inset). Spleen and lung tissues were also stained with isotype-matched irrelevant Abs (D and E, insets, respectively).

**FIGURE 4.** CD14 and CD89 mRNA expression in lamina propria macrophages and blood monocytes. Fresh blood monocytes purified from two donors and lamina propria macrophages purified from four donors were analyzed by RT-PCR for CD14, CD89, and GAPDH (control) mRNA transcripts. Shown are the electrophoretic patterns of PCR products on a 2% agarose gel stained with ethidium bromide.

**FIGURE 5.** TLR2 and TLR4 mRNA expression in lamina propria macrophages. Lamina propria macrophages purified from normal jejunum from three donors and control THP-1 cell line macrophages (American Type Culture Collection, Manassas, VA) were analyzed by RT-PCR for TLR2, TLR4, and GAPDH (control) mRNA transcripts. Shown are the electrophoretic patterns of PCR products on a 2% agarose gel stained with ethidium bromide.
shown in Fig. 7, the blockade of surface CD14 caused a dose-dependent decrease in LPS-stimulated IL-8 production, supporting the concept that the absence of CD14 on lamina propria macrophages could account for the down-regulation of CD14-mediated effector function.

Consistent with their other macrophage features (7, 10), the lamina propria macrophages exhibited avid phagocytic activity for BSA-coated beads (Fig. 8). When the beads were coated with LPS or IgA to assess CD14- and CD89-mediated phagocytosis, monocyte phagocytosis increased 2-fold, in keeping with the presence of surface CD14 and CD89. In contrast, phagocytosis by CD14−CD89− intestinal macrophages decreased, possibly due to LPS and IgA blocking the BSA ligands recognized by other phagocytosis receptors.

Discussion

We report here that resident macrophages isolated from the lamina propria of normal human intestine do not express surface CD14 or CD89 and, consequently, do not perform CD14 or CD89 receptor-mediated functions, such as LPS-stimulated cytokine production and LPS- and IgA-enhanced phagocytosis. The absence of CD14 and CD89 on the macrophages was not due to the isolation procedure, because lamina propria macrophages in situ were CD14+CD89+, and similarly treated monocytes remained CD14+CD89+. The absence of CD14 and CD89 appeared not to be due to digestion by products of other mucosal cells, because 24-h exposure of monocytes to mucosal cell-conditioned medium did not down-modulate the receptors, and culture of lamina propria macrophages in the absence of mucosal factors did not induce CD14 or CD89 expression. Studies are currently underway to determine whether longer exposure to mucosal cell products affects surface receptor expression. Importantly, lamina propria macrophages expressed neither CD14 nor CD89 mRNAs, implicating down-regulated gene transcription in the etiology of the CD14−CD89− phenotype.

Our findings indicate that lamina propria macrophages express mRNA for TLR2 and TLR4, protein receptors that induce a signaling pathway that leads to cytokine gene transcription (9, 26, 27). Although we have not yet examined intestinal macrophages for defects in the TLR signaling pathway, the presence of TLR mRNAs in lamina propria macrophages suggests that the unresponsiveness of the cells to LPS is not due to down-regulated gene transcription for TLR2 and TLR4 (28). In contrast, the dose-dependent decrease in IL-8 production by blood monocytes preincubated with anti-CD14 Abs supports the idea that the LPS unresponsiveness of lamina propria macrophages is due to the absence of CD14. The inability of lamina propria macrophages to mount an enhanced phagocytic response to beads coated with LPS, a response that is not mediated by TLRs, supports the idea that the absence of CD14 is the cause of the LPS unresponsiveness. Although previous investigators have shown that soluble CD14 plus LPS can activate monocytes and neutrophils (29), the monocytes in that study expressed membrane-bound CD14 (mCD14). Taken together, our findings strongly implicate the absence of mCD14 in the markedly reduced response of lamina propria macrophages to LPS.

The absence of mCD14 on lamina propria macrophages and the consequent down-regulation of LPS-induced inflammatory responses offer an explanation for the low level of inflammation in normal intestinal mucosa despite the close proximity of bacteria and bacterial LPS. However, during infectious and inflammatory processes in the intestinal mucosa, CD14+ blood monocytes are probably recruited to the mucosa (5, 6) where, presumably in response to local stimulatory signals, they produce proinflammatory mediators that promote tissue inflammation (30, 31). Although the results presented here pertain to jejunal lamina propria macrophages, evidence suggests that our results may be relevant to the colon where lamina propria macrophages also appear to lack CD14 (6, 8).

FIGURE 6. LPS-stimulated IL-8 production by lamina propria macrophages and blood monocytes. Purified lamina propria macrophages and blood monocytes were cultured in the presence of medium alone or with the indicated concentrations of LPS in the absence or the presence of 10% human AB serum as a source of LPS-binding protein, and 24-h culture supernatants were analyzed for IL-8 by ELISA. Data are representative of three separate experiments.

FIGURE 7. Anti-CD14 Ab blockade of IL-8 production by LPS-stimulated blood monocytes. Blood monocytes were preincubated with anti-CD14 (MEM-18) Ab at the indicated concentrations for 2 h, washed, and then incubated for 24 h with LPS 1 μg/ml, after which the amount of IL-8 in the supernatants was quantitated by ELISA (n = 3).

FIGURE 8. Phagocytic activity of lamina propria macrophages and blood monocytes. Purified lamina propria macrophages and blood monocytes were incubated for 1 h in the presence of 10% human AB serum with fluorescence-labeled beads coated with BSA (A), LPS followed by BSA (B), or IgA followed by BSA (C) and then analyzed by flow cytometry to determine the level of phagocytosis of the beads. Data are representative of two separate experiments.
Consistent with their central role in host defense against intrin
tuber material and micro-organisms that have crossed the epithelium,
alamina propria macrophages are strongly phagocytic for inert
beads, as we have shown here, and for live micro-organisms (7).
Indeed, the spontaneous phagocytic activity of lamina propria
macrophages for the beads was greater than that of blood mono-
cytes. However, the absence of CD89 on lamina propria macrho-
dages down-regulates IgA-mediated phagocytosis, an activity
that normally releases the defense of proinflammatory mediators,
including reactive oxygen intermediates, leukotrienes, and PGs
(32, 33). Thus, the absence of CD89 on intestinal macrophages
further down-modulates mucosal inflammatory responses. In
addition, secretory IgA is largely the product of local synthesis at the
mucosal surface, where the dimeric forms of IgA produced by
lamina propria plasma cells are transported across the mucosal
epithelium by poly Ig receptor-mediated transcytosis (34). These
secretory IgA Abs play an important protective role against envi-
ronmental pathogens and Ags encountered at the mucosal surface
(35). Thus, the absence of CD89 on lamina propria macrophages is
in accordance with normal physiology, because secretory IgA is not
"hijacked" by lamina propria macrophages before reaching the
epithelial cells.

Our findings that intestinal lamina propria macrophages are pha-
notypically, functionally, and genotypically distinct from CD14+
macrophages from peripheral blood monocytes, including the ab-
sence of surface FcεR and FcyRII-III, down-regulated inducible
cytokine production, and absent CD14 and CD89 transcripts
(Smythies et al., manuscript in preparation), suggesting that intes-
tinal macrophages are derived from CD14+ blood monocytes
(32, 33). Thus, the absence of CD89 on intestinal macrophages
likely hijacks lamina propria macrophages before reaching the
epithelial cells.

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