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Intestinal Macrophages Lack CD14 and CD89 and Consequently Are Down-Regulated for LPS- and IgA-Mediated Activities¹

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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 receptor for LPS and CD89, the receptor for IgA (FcγR). 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.


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 countercflow 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 release the lamina propria mononuclear cells. After gradient sedimentation, the macrophages were purified by elutriation (10). Additional tissue was formalin-fixed and paraffin-embedded, and 5-μm sections were stained with macrophage-specific mAb HAM56 (DAKO, Carpinteria, CA) and examined by light microscopy. For ultrastructural analysis, isolated macrophages were examined by electron microscopy as previously described (7). Blood monocytes were purified from healthy donors by elutriation (10) and then treated with dispase according to the macrophage isolation protocol so that both cell populations were treated similarly. The cells isolated by these techniques were routinely 100% viable by propidium iodide staining.

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

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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.
serum (Coulter, Miami, FL) plus 10% normal mouse serum (15 min, 22°C; Jackson ImmunoResearch Laboratories, West Grove, PA) and then stained with PE-conjugated mouse mAbs to either CD13 (aminopeptidase N, present exclusively on mononuclear phagocytes and epithelial cells; 25 μg/ml; BD Pharmingen, San Jose, CA) or CD14 (LeuM3; 25 μg/ml; BD Pharmingen) and then, after washing, with FITC-conjugated mAbs to either HLA-DR (25 μg/ml; BD Pharmingen) or CD89 (100 μg/ml) (12, 13). Control Abs included PE- or FITC-labeled irrelevant mouse mAb of the same IgG isotype. After staining, cells were fixed, in 1% paraformaldehyde, and analyzed by FACS. Data were analyzed with CellQuest software (BD Biosciences, Mountain View, CA).

To assess the purity of the isolated lamina propria macrophages, cells were incubated with PerCP-CD3 (100 μg/ml), PE-CD11b (50 μg/ml), PE-CD11c (12.5 μg/ml), PE-CD14 (25 μg/ml), PE-CD34 (25 μg/ml), PE-CD123 (0.025 μg/ml; all from BD Pharmingen), FITC-CD21 (Accurate Chemical and Scientific, Westbury, NY), FITC-CD103 (25 μg/ml) and PE-CD28 (25 μg/ml; both from Immunotech, Westbrook, ME), or control PerCP-, PE-, or FITC-labeled, isotype-matched, irrelevant mAbs (10 μg/ml) and analyzed by flow cytometry as described above. To assess the effect of dispase on the level of surface CD14 and CD89, dispase-treated monocytes were analyzed by FACS for CD14 with MO2 (2.5 μg/ml) and MO4 (0.625 μg/ml; Coulter Immunology, Hialeah, FL), FMC17 (100 μg/ml), TUK4 (200 μg/ml), YMEM18 (200 μg/ml), UCHM-1 (50 μg/ml), and FMC-32/2D-15C (100 μg/ml; Accurate Chemical and Scientific) and for CD89 (100 μg/ml) with A62, A77, and MY43 each 10 μg/ml, as described above (12–15).

**Immunohistochemical analysis of tissue specimens for CD14** and CD89** cells**

Blocks of small intestine, lung, and spleen tissue from the same donor were snap-frozen in embedding medium (OCT; Sakura Fine Tek, Torrance, CA) and maintained at −70°C. Frozen sections (4 μm) were placed on electrostatically charged slides (SuperFrost Plus; Fischer Scientific, Pittsburgh, PA) and fixed by irradiation in 0.5% paraformaldehyde/5% Kryofix in 0.1 M phosphate-buffered saline, pH 7.4, for 60 s at 650 W. Serial sections were rinsed in PBS, incubated in 3% hydrogen peroxide (45 min) to block endogenous peroxidase activity, exposed to casein protein (1 h, CSA System; Dako) to block nonspecific background, and incubated with primary mouse anti-human anti-CD14 (0.05 mg/ml, 30 min; BD Pharmingen), anti-CD89 (0.05 mg/ml, 30 min; BD Pharmingen), HAM56 (0.05 mg/ml, 10 min; Dako), or control Abs (mouse IgG2a and IgG1; BD Pharmingen and Dako). Sections were then washed in PBS, incubated with anti-mouse labeled polymer (HRP), followed by diaminobenzidine-positive AB’ substrate-chromogen solution (EnVision+ System; Dako), and counterstained with methyl green.

**RT-PCR**

Extracted RNA was reverse transcribed into first-strand cDNA (11), and the cDNA (5 μl) was amplified in a 50-μl reaction containing 0.25 μl Taq polymerase (5 U/ml, Gene Amp PCR system; PerkinElmer/Cetus, Norwalk, CT), 4 μl dNTPs (2.5 mmol/l; Promega, Madison, WI), 2 μl MgCl2 (25 mmol/l), 5 μl 10× PCR buffer, and 2.5 μl each of primers for CD14 (5′-GTCCTGGACCTTGGACAAACG (antisense) and 5′-CCATGGAACGC CGGTCCTGCG (sense)), CD89 (5′-TGAGCTGCACGTGAGGGAAAT (antisense) and 5′-CAAGGAAAGGGACTTCTTCAT (sense)), TLR2 (5′-GGCAAAGTGTGATGTTGG (antisense) and 5′-TTGAAAGTTTCCTCA GCTCTCG (sense)), and TLR4 (5′-TGGATACGTTTCCTTATAAG (antisense) and 5′-GGAAATGGACGCACCTTTC (sense)) (16). The reaction mixture was amplified (CD14: denaturation at 95°C for 1 min, annealing at 62°C for 2 min, extension at 72°C for 3 min, 30 cycles; CD89: denaturation at 95°C for 1 min, annealing at 58°C for 2 min, extension at 72°C for 3 min, 30 cycles; TLR2 and TLR4: denaturation at 96°C for 40 s, annealing at 54°C for 40 s, extension at 72°C for 1 min, 35 cycles), and the products were detected by gel electrophoresis.

**Macrophage function assays**

CD14 and CD89 receptor-mediated macrophage function was evaluated by quantitating LPS-stimulated IL-8 production and phagocytic activity for LPS- and IgA-coated beads. Lamina propria macrophages and blood monocytes (2 × 10^6/ml) were incubated for 24 h in DMEM in the absence or the presence of LPS (Sigma, St. Louis, MO) with or without 10% human AB serum (Atlanta Biologicals, Atlanta, GA) as a source of LPS-binding protein, and the amount of IL-8 released was measured by ELISA (R&D Systems, Minneapolis, MN).

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 HLA-DR 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 CD5 and CD103.

Intestinal lamina propria macrophages were analyzed in parallel with purified blood monocytes for surface CD14, CD89, CD13, and HLA-DR. Greater than 98% of the lamina propria macrophages and blood monocytes expressed CD13 and HLA-DR (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

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**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 jejenum (×32).
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 HAM56⁺ 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 mRNA. 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.

### Table 1. Effect of mucosal cell-conditioned media on monocyte surface CD14 and CD89 expression

<table>
<thead>
<tr>
<th>Mucosal Cell-Conditioned Medium</th>
<th>Ab</th>
<th>Medium</th>
<th>Epithelium</th>
<th>Mononuclear cells</th>
<th>Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14 (LeuM3)</td>
<td>73</td>
<td>84</td>
<td>80</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>CD89 (A59)</td>
<td>71</td>
<td>82</td>
<td>85</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>95</td>
<td>99</td>
<td>97</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage of ungated cells.*
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...
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 offers 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).
Consistent with their central role in host defense against inert material and micro-organisms that have crossed the epithelium, lamina 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 monocytes. However, the absence of CD89 on lamina propria macrophages down-modulates IgA-mediated phagocytosis, an activity that normally induces the release of inflammatory 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 environmental 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 pheno-

typically, functionally, and genotypically distinct from CD14+

blood monocytes were unexpected. However, the absence of

CD14, one of the most important and primitive pattern recognition

molecules of the innate immune system, offers the species distinct

selection advantages, namely, down-modulation of potentially

harmful inflammation at the body site richest in proinflammatory

signals. In this regard, preliminary evidence indicates that intesti-
nal macrophages share many phenotypic, functional, and transcrip-
tional features with CD14− blood monocytes, including the ab-

cence of surface FcRf and FcγRf-II, down-regulated inducible

cytokine production, and absent CD14 and CD89 transcripts

(Smythies et al., manuscript in preparation), suggesting that intesti-
nal macrophages are derived from CD14− blood monocytes (~5% of the circulating monocyte population) and that CD14−

blood monocytes are preferentially recruited to the intestinal mu-
cosa where they populate the lamina propria. In summary, lamina propria CD14− CD89− macrophages are a unique population of mononuclear phagocytes whose down-modulated proinflammatory activities probably contribute substantially to maintaining the low level of inflammation in normal human intestinal mucosa.

References


macrophage content in adult mouse tissues: immunochromchemical studies with mono-


