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*J Immunol* 2009; 183:1724-1731; Prepublished online 10 July 2009; doi: 10.4049/jimmunol.0804369

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Human CD14⁺ Macrophages in Intestinal Lamina Propria Exhibit Potent Antigen-Presenting Ability

Nobuhiko Kamada,* Tadakazu Hisamatsu,† Haruki Honda,* Taku Kobayashi,* Hiroshi Chinen,* Mina Tokutake Kitazume,* Tetsuro Takayama,* Susumu Okamoto,* Kazutaka Koganei,† Akira Sugita,† Takanori Kanai,* and Toshifumi Hibi2*  

Intestinal APCs are considered critical in maintaining the balance between the response against harmful pathogens and the induction of tolerance to commensal bacteria and food Ags. Recently, several studies indicated the presence of gut-specific APC subsets, which possess both macrophage and dendritic cell (DC) markers. These unique APC subsets play important roles in gut immunity, especially for immune regulation against commensal bacteria. Herein, we examined a unique macrophage subset, which coexpressed the macrophage (Mφ) marker CD14 and the DC marker CD209 in human intestinal lamina propria (LP). The LP Mφ subset in both normal control subjects or Crohn’s disease (CD) patients induced proliferation of naive CD4⁺ T cells as well as monocyte-derived DCs, and it expressed retinoic acid synthetic enzyme retinaldehyde dehydrogenase 2 and retinol dehydrogenase 10, which induced expression of gut homing receptors on T cells in a retinoic acid-dependent manner. Moreover, the LP Mφ subset strongly evoked differentiation of Th1 cells and slightly induced Th17 cells in both normal control subjects and CD patients; the inducing potential was highest in CD patients. In CD patients, Th17, but not Th1, induction by the LP Mφ subset was enhanced in the presence of commensal bacteria Ags. This enhancement was not observed in normal control subjects. The Th17 induction by the LP Mφ subset was inhibited by neutralization of IL-6 and IL-1β, but it was enhanced by blockade of retinoic acid signaling. These observations highlight a role for LP Mφ in the enhanced Th1 and potentially in Th17 differentiation, at the inflammatory site of inflammatory bowel diseases. The Journal of Immunology, 2009, 183: 1724–1731.

The intestinal immune system faces two conflicting tasks in that it needs to respond robustly against harmful pathogens, but remain immune tolerant to commensal bacteria and food Ags. APCs such as dendritic cells (DCs) and macrophages (Mφs) are thought to be critical in maintaining this intestinal immune system balance (1). Recent studies have demonstrated antiinflammatory roles of intestinal Mφs (2, 3). Human intestinal Mφs do not express innate response receptors, and although these cells retain their phagocytic and bactericidal functions, they do not produce proinflammatory cytokines in response to several inflammatory stimuli, including microbial components (4, 5). Additionally, recent studies revealed that intestinal Mφs express several antiinflammatory molecules, including IL-10 (6), and induce the differentiation of Foxp3⁺ regulatory T cells (Tregs) by a mechanism dependent on IL-10 and retinoic acid (RA) (7). Thus, Mφs located in the intestinal mucosa are considered to play important roles in the maintenance of intestinal homeostasis by protecting the host from foreign pathogens and by negatively regulating excessive immune responses to commensals.

On the other hand, abnormal immune responses against commensals are considered a key pathogenic factor in development of chronic intestinal inflammation such as inflammatory bowel disease (IBD) (8–10). Indeed, it has been intensively reported that disturbed antiinflammatory functions of intestinal Mφs may cause abnormal immune responses to commensals and may lead to the development of chronic intestinal inflammation (6, 11–13). We have previously reported that intestinal CD14⁺ Mφs play a central role in the pathogenesis of human Crohn’s disease (CD), a major form of IBD, via excess production of IL-23 and TNF-α in response to commensal bacteria (14). Interestingly, this intestinal Mφ subset expresses some DC markers, including CD209, on their cell surface, as well as Mφ markers. However, it remains unclear whether this Mφ subset has any DC functions such as Ag presentation to naive T cells. In the present study, we focused on the DC-like functions of this intestinal Mφ subset and examined their role in the pathogenesis of IBD, particularly in CD.

Materials and Methods

Tissue samples

Normal intestinal mucosa was obtained from macroscopically and microscopically unaffected areas in patients with colon cancer. Intestinal mucosa was obtained from surgically resected specimens from patients with CD and diagnosed on the basis of clinical, radiographic, endoscopic, and histological findings, according to established criteria. In all samples from patients with CD, the degree of inflammation was histologically moderate to severe. All experiments were approved by the Institutional Review Board of Yokohama Municipal Citizen’s Hospital, Yokohama, Japan; Keio University School of Medicine, Tokyo, Japan; and Department of Surgery, Keio University Medical Science Fund, Keio Ishikai Fund, and Japanese Foundation for Applied Enzymology.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.0804369

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Received for publication December 31, 2008. Accepted for publication May 25, 2009.

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1 This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan Society for the Promotion of Science, Keio University Research Grants for Life Science and Medicine, Keio University Medical Science Fund, Keio Ishikai Fund, and Japanese Foundation for Applied Enzymology.

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3 Abbreviations used in this paper: DC, dendritic cell; Mφ, macrophage; Treg, regulatory T cell; RA, retinoic acid; IBD, inflammatory bowel disease; CD, Crohn’s disease; Mo-Mφ, monocyte-derived macrophage; Mo-DC, monocyte-derived dendritic cell; PB, peripheral blood; LPMC, lamina propria mononuclear cell; LP, lamina propria; NC, normal control; RALDH, retinaldehyde dehydrogenase; RDH, retinol dehydrogenase.
FIGURE 1. LP CD14⁺ CD209⁺ Mds in the human intestine reveal potent Ag presenting ability. A. Fluorescence microscopy of human intestine from CD patients stained with anti-CD14 (green) and anti-CD209 (red). CD14⁺ CD209⁺ Mds were present in the intestinal LP. Data are representative of three independent experiments. B. Giemsa staining of isolated LP-Mds from NC and CD patients. Scale bar, 20 μm. C. Purified LP CD14⁺ cells from NC and CD patients (LP-Mδ), Mo-DCs, and Mo-Mδs were cocultured with CFSE-labeled naive CD4⁺ T cells for 6 days at a 10:1 ratio. Proliferated cells were determined as CFSE low expression cells. D. Expression of the memory T cell marker CD45RO Ags following allogeneic MLR with APCs from the gut and in vitro-generated APCs, respectively. E. Percentages of proliferated cells were determined as CFSE low expression cells. Data represent means ± SEM from individual patients or controls (Mo-DCs, n = 9; Mo-Mδs, n = 9; LP-Mδs (NC), n = 6; LP-Mδs (CD), n = 7). Statistical analysis was performed by Kruskal-Wallis one-way ANOVA and Tukey-Kramer test for multiple comparisons. *** p < 0.001.

Board of Keio University School of Medicine and Yokohama Municipal Citizen’s Hospital, and written informed consent was obtained from all patients.

Histological analysis

Tissue sections were treated according to well-established methods. Intestinal specimens were embedded in TissueTek OCT compound (Sakura Finetek), snap frozen in liquid nitrogen, and sectioned using a cryostat. Sections 5 μm thick were fixed with ice-cold acetone (Wako Pure Chemical Industries), treated with 3% hydrogen peroxide (Wako Pure Chemical Industries) in 100% methanol, and then incubated with normal rabbit serum (Nichirei Biosciences) in 100% methanol, and then incubated with normal rabbit serum (Nichirei Biosciences) for 15 min at room temperature to block nonspecific reactions. Thereafter, sections were incubated with mouse anti-human CD14 Ab (Zymed Laboratories) at 4°C overnight. After washing with PBS, the sections were incubated with Alexa Fluor 488-conjugated secondary Ab (Molecular Probes). For double labeling, slides were boiled for 15 min in 0.01 M citrate buffer (pH 6.4; Wako Pure Chemical Industries) and were observed with a light microscope.

Isolation of lamina propria (LP) Mδs

LP CD14⁺ Mδs were isolated from LPMCs using EasySep human CD14⁺ (StemCell Technologies). The percentage of each subset of cells isolated using this method was evaluated by flow cytometry and was routinely ~95%.

Giemsa staining

Isolated LP CD14⁺ Mδs were spread on glass slides and were air-dried. The spread cells were then fixed with methanol and stained with Giemsa solution (pH 6.4; Wako Pure Chemical Industries) and were observed with a light microscope.

Commensal bacteria heat-inactivated Ags

A Gram-positive commensal strain of Enterococcus faecalis (American Type Culture Collection 29212) was cultured in brain-heart infusion medium. Bacteria were harvested and washed twice with ice-cold PBS. Next, bacterial suspensions were heated at 80°C for 30 min, washed, resuspended in PBS, and stored at −80°C. Complete killing was confirmed by 72 h of incubation at 37°C on plate medium.

Cell proliferation assay

CD45RO CD45RA⁺ naïve CD4⁺ T cells were isolated from PBMCs of healthy volunteers using naïve CD4⁺ T cell MACS (Miltenyi Biotec). Isolated peripheral blood naïve CD4⁺ T cells were stained with 5 μM CFSE (Molecular Probes) for 10 min at 37°C. Labeled cells were cultured for 6 days for naïve T cell differentiation and analyzed by flow cytometry.

Mixed leukocyte reaction

To perform the allogeneic MLR, isolated naïve CD4⁺ T cells (1 × 10⁵ cells/ml) were cocultured with isolated LP CD14⁺ Mδs (1 × 10⁵ cells/ml) from normal control subjects (NC) or CD patients for 6 days at a ratio of 10:1 in round-bottom 96-well culture plates in the presence or absence of heat-inactivated E. faecalis (1 × 10⁵ CFU/ml). After 6 days, T cells were isolated using CD3⁺ iMag (BD Pharmingen) and rested for 2–5 days with...
FIGURE 2. RA derived from LP Mφs promotes the imprinting of gut-homing ability on T cells. A. Expression of gut-homing receptors on differentiated naive CD4+ T cells by the coculture with LP CD14+ cells from NC or CD patients or in vitro-differentiated naive CD4+ T cells by anti-CD3/CD28 Abs. Data represent means ± SEM of the expression of indicated gut-homing markers gated by CD4+CD45RO+ memory T cells (in vitro T, n = 6; LP CD14+ (NC), n = 4; LP CD14+ (CD), n = 5). B. Expression of gut-homing receptors on CD4+CD45RO+ memory T cells cultured with LP CD14+ cells in the presence (LE540) or absence (Medium) of an RAR antagonist LE540 (1 μM). In vitro-differentiated CD4+CD45RO+ memory T cells by anti-CD3/CD28 Abs were used as a control. Data are representative of at least four independent experiments. Statistical analysis was performed by Kruskal-Wallis one-way ANOVA and Tukey-Kramer test for multiple comparisons. *p < 0.05 and ***, p < 0.001.

Quantitative real-time RT-PCR analysis

Total RNA was extracted using an RNeasy Micro kit (Qiagen), and cDNA was synthesized using a QuantiTect Reverse Transcription Kit (Qiagen). Total RNA was extracted using an RNeasy Micro kit (Qiagen), and cDNA was synthesized using a QuantiTect Reverse Transcription Kit (Qiagen). For real-time RT-PCR, we performed qPCR using the CFX96 Real-Time System or ABI PRISM 7900HT Instrument (both Applied Biosystems). Relative quantification was normalized by the value of the β-actin (ACTB) (Hs99999903_m1) gene.

Cytokine assay

The following kits were used for cytokine measurements and were performed according to the manufacturer’s instructions: human IL-17 ELISA (R&D Systems), human IL-23 ELISA (R&D), and human inflammation and Th1/Th2-II cytokometric beads array kit (BD Pharmingen).
CD45RA⁺CD45RO⁻ naive CD4⁺ T cells were isolated from peripheral blood of a healthy volunteer and labeled with CFSE. Naive CD4⁺ T cells did not proliferate during the culture period without any stimulation. Thus, the CFSE fluorescence intensity remained high (Fig. 1C). Of note, LP CD14⁺ Mφs from both NC and CD groups induced naive CD4⁺ T cell proliferation. The percentage of proliferated CD4⁺ T cells and induced CD4⁺CD45RO⁺ T cells by LP CD14⁺ Mφs from both NC and CD groups were comparable to those by Mo-DCs and were significantly higher than those by Mo-Mφs (Fig. 1, D and E). These data suggest that LP CD14⁺ Mφs can induce proliferation of naive T cells, as well as Mo-DCs, but with no significant difference between NC and CD patients.

LP CD14⁺ Mφs induced gut-tropic CD4⁺ T cells via a RA signaling

Several recent studies have demonstrated that APCs from gut-related tissues express the RA metabolic enzyme retinaldehyde dehydrogenase (RALDH)2, and were able to generate RA (16, 17). RA induces gut-homing marker expression on T cells during Ag presentation and also contributes to the differentiation of Tregs (16, 18). Thus, RA-mediated education of T cells plays important roles in gut-specific immune responses.

In this experiment, we examined the imprinting function of LP CD14⁺ Mφs. In vitro-differentiated CD45RO⁺ memory CD4⁺ T cells in a nonskewed condition exhibited surface integrin α₄ expression, but only expressed limited levels of integrin β₇ and CCR9 (Fig. 2A). Naive CD4⁺ T cells did not express these markers (data not shown). On the other hand, LP CD14⁺ Mφs induced expression of CD45RO⁺ memory CD4⁺ T cells and higher levels of integrin β₇ (Fig. 2A). CCR9 expression was higher on T cells differentiated by LP CD14⁺ Mφs than on in vitro-differentiated T cells, although CCR9⁺ cells were <5% of CD4⁺CD45RO⁻ memory T cells (Fig. 2A). Thus, it was difficult to conclude whether LP CD14⁺ Mφs induced functional CCR9 on naive CD4⁺ T cells. Additionally, the gut-tropic T cell-inducing capacity of LP CD14⁺ Mφs was not different between NC and CD patients (Fig. 2A).

As described above, RA generated by gut-specific APCs is an essential factor for induction of gut-homing marker α₄β₇ integrin and CCR9 on T cells. Therefore, we tested the role of the RA signaling in the induction of α₄β₇ integrin by LP CD14⁺ Mφs. As expected, treatment with LE540, an RAα,β antagonist, clearly suppressed the induction of integrin β₇ by LP CD14⁺ Mφs (Fig. 2B). Unexpectedly, surface expression of CCR9 was not affected (Fig. 2A). This RA-dependent induction of β₇ integrin by LP CD14⁺ Mφs was observed similarly in NC and CD groups (data not shown). These data suggest that LP CD14⁺ Mφs in both NC and CD patients induce the expression of β₇ integrin on naive CD4⁺ T cells in a RA-dependent manner.

LP CD14⁺ Mφs induced helper T cell differentiation

Next, we examined the ability of helper T cell differentiation by LP CD14⁺ Mφs. Proliferated CD4⁺ T cells by MLR experiments were restimulated, and production of Th1 and Th17 cytokines was analyzed by intracellular cytokine staining. As shown in Fig. 3, A and B, LP CD14⁺ Mφs in both NC and CD groups induced robustly IFN-γ-positive CD4⁺ T cells (Th1 cells) and slightly IL-17-positive CD4⁺ T cells (Th17 cells), while Mo-DCs did not induce Th17 cells. The inducing ability of both Th1 and Th17 cells was modestly higher in LP CD14⁺ Mφs in CD patients than those in NC (Fig. 3B). In Mo-DCs, IFN-γ, but not IL-17, secretion was observed in the supernatant of APC-T cell cocultures (Fig. 3C).
Although a detectable number of Th17 cells were induced by LP CD14+ Mφs from NC, the IL-17 secretion in coculture supernatants was almost undetectable (Fig. 3C). On the other hand, consistent with the results from intracellular cytokine staining, high levels of both IFN-γ and IL-17 were observed in the culture supernatants of naive CD4+ T cells cocultured with LP CD14+ Mφs from CD patients (Fig. 3C). Interestingly, the presence of the commensal bacteria *E. faecalis* during MLR enhanced the differentiation of Th17 cells, but not Th1 cells, in MLR with LP CD14+ Mφs from CD patients (Fig. 3C). In contrast, only differentiation of Th1 cells, but not Th17 cells, was enhanced by the presence of *E. faecalis* in Mo-DCs-MLR (Fig. 4A–C). In the NC group, although both Th1 and Th17 induction were observed by MLR, an enhancement of Th17 by commensal bacteria was not identified. Additionally, either IL-17 production in MLR culture or transcription of IL-17 mRNA levels in the proliferated CD4+ T cells by allogeneic MLR was enhanced in the presence of commensal bacteria when the LP CD14+ Mφs from CD patients were used as an APC (Fig. 4, D and E). These data suggest that the LP CD14+ Mφs in CD patients exhibit the potential to induce robust Th1 immunity and possible Th17 immunity, while the presence of commensal bacteria elevates the Th17, but not Th1, induction.

LP CD14+ Mφs induced Th17 differentiation via IL-6 and IL-1β, but not IL-23

Next, we tried to identify the key regulator of Th17 induction by the LP CD14+ Mφs from CD patients. First, we examined the APC-derived cytokines from MLR culture supernatants. As shown in Fig. 5A, robust amounts of IL-6, IL-1β, and IL-23 were detected in MLR supernatants cocultured with LP CD14+ Mφs, particularly in CD patients. On the other hand, Mo-DC coculture contained only limited amounts of these cytokines, but a large amount of IL-12p70, an important cytokine inducing Th1 immunity (Fig. 5A). As we have previously reported the importance of IL-23 derived from LP CD14+ Mφs in the pathogenesis of CD, we first focused on the role of IL-23 in Th17 induction by LP CD14+ Mφs. However, IL-23 did not affect Th17 induction (Fig. 5B). Moreover, neutralizing IL-23 during LP CD14+ Mφs-MLR suppressed only Th1 induction, but did not affect Th17 induction (Fig. 5C).

In addition to IL-23, several studies have reported that IL-6 and IL-1β are important cytokines for driving Th17 cells in humans (19, 20). Consistent with previous reports, in the present study the Th17 induction was dramatically suppressed by neutralization of
IL-6 and IL-1β (Fig. 5D). These data suggest that LP CD14- Mφ-derived IL-6 and IL-1β, but not IL-23, evoke the differentiation of Th17 cells from naive T cells in the intestine.

**RA signaling is important in suppression of Th17-driving by LP Mφs, and RA signaling may be impaired in LP Mφs in CD patients**

As demonstrated, LP CD14- Mφ-derived IL-6 and IL-1β are important regulators of Th17 polarization. To determine the mechanisms by which these cytokines induced Th17 differentiation, we focused on the role of RA in Th17 differentiation. As previously reported, TGF-β is an essential factor for inducing Treg differentiation, while TGF-β also induces Th17 differentiation in the presence of IL-6 (18). RA was reported to induce Treg differentiation and inhibit Th17 differentiation by TGF-β. Consistent with previous reports, in the present study suppression of RA signaling by a RAR antagonist, LE540, up-regulated the differentiation of Th17 cells (Fig. 6, A and B). Moreover, blockade of RA signaling enhanced the production and transcription of IL-17, but not IFN-γ, in CD4+ T cells cocultured with LP CD14+ Mφs (Fig. 6, C and D). These data suggest that RA signaling acts as a suppressor of Th17-driving by LP CD14+ Mφs.

In the present study, the Th17-inducing ability of LP CD14+ Mφs was markedly enhanced in CD patients compared with NC (Figs. 3 and 4). These results might suggest the possibility that RA-dependent suppression pathway was impaired in LP CD14+ Mφs of CD patients. To address this point, we examined the expression of RALDH2 and retinol dehydrogenase (RDH)10 in LP CD14+ Mφs. Consistent with previous reports, normal LP CD14+ Mφs expressed higher levels of RALDH2, a RA synthesis enzyme that converts retinol to all-trans RA, when compared with peripheral blood BDCA-1+ DCs (PB-DCs) (Fig. 6E). Unexpectedly, RALDH2 expression was also higher in Mo-DCs (Fig. 6E). However, expression of RDH10, an RA synthesis enzyme that converts retinol to retinol, was obviously higher only in NC LP CD14+ Mφs (Fig. 6E). These data suggest that LP CD14+ Mφs in normal intestine generate RA from retinol more efficiently than with peripheral DCs. Of note, RDH10 expression in LP CD14+ Mφs was significantly lower in CD patients than in NC, while a similar level of RALDH2 expression was observed in both groups (Fig. 6E). Thus, RA generation by LP CD14+ Mφs might be impaired in patients with CD, so that Th17 induction by LP CD14+ Mφs of CD patients was potentiated compared with NC.

**Discussion**

Several studies have identified unique subsets of APCs that possess both DC and Mφ markers in intestinal LP (21). These subsets may contribute to both homeostatic maintenance of intestine and the pathogenesis of intestinal inflammation. For example, a subset of CD11cintCD11bhi LP DCs in the murine small intestine co-expressed the Mφ marker F4/80 and induced differentiation of naive T or B cells toward Th1 and Th17 cells or IgA-producing plasma cells (22). In that study, IL-6 production by LP DCs via TLR5 was important for Th17 induction. In addition to this subset, a recent study showed that a subset of CD11cintCD79a+CD4+CD123+ LP DCs in the mouse intestine preferentially drives Th17 cells in the presence of commensal-derived ATPs (23). Moreover, another subset of murine intestinal DCs that coexpressed the Mφ marker F4/80 directly induced the development of granuloma dependent on commensal bacteria-induced IL-23 (24). Thus, these unique APC subsets that have both DC and Mφ markers play important roles in gut immunity, especially for the immune regulation against commensal bacteria. In the present study, we also identified such an “intermediate” APC subset in the human intestine. We previously demonstrated that CD14-CD209+ LP Mφs were significantly increased in IBD patients, particularly in those patients with CD, and that this subset might be involved in the pathogenesis of CD via production of IL-23 (14). In addition to these data, in the present study we determined the Ag presenting function of this LP Mφ subset. The LP CD14+ Mφ subset in either NC or CD patients induced predominantly Th1 cell differentiation. Although Th17 induction by the LP CD14+ Mφ subset was weaker than that of Th1, the LP CD14+ Mφ subset induced a detectable amount of Th17 cells. Moreover, LP CD14+ Mφs from CD patients were a more potent inducer of both Th1 and Th17 than those from NC.

Interestingly, we observed that the Th17 induction by LP CD14+ Mφs from CD patients was enhanced in the presence of commensal bacteria, while Th1 was not. Similarly, intracellular bacteria recognition receptor NOD2-mediated IL-23 and IL-1β production has been shown to be important for Th17 induction by...
human DCs (25), while we reported that commensal bacteria induce IL-23 production by LP CD14+ Møs via intracellular bacteria recognition (14). On the other hand, in the present study, IL-23 was not involved in the Th17 induction from naive CD4+ T cells. Additionally, we previously demonstrated that commensal bacteria-stimulated LP Møs enhanced the production of IFN-γ, but not IL-17, from LP T cells via IL-23 in CD patients (14, 26), implying that the LP Møs induce a Th1 immune response rather than Th17 on “memory” CD4+ T cells through IL-23, while LP Møs may induce the differentiation of “naive” CD4+ T cells toward both Th1 and Th17 immunity, but not via IL-23.

With respect to the mechanism of induction of Th17 differentiation by LP CD14+ cells, in the present study, IL-6 and IL-1β were important for Th17 differentiation from naive CD4+ T cells by LP CD14+ Møs. Higher amounts of these cytokines were detected in MLR culture supernatants of LP Møs than in those of Mo-DCs. Furthermore, LP Møs in CD patients produced higher cytokine amounts than those in NC.

It remains unclear whether the intermediate APC subset is a subset of Møs or of DCs. A similar intermediate APC subset was reported in other human tissues. For example, CD14+CD1a- and CD14+CD1a+ dermal APCs were present in human skin, and the CD14+ dermal APCs also expressed some DC markers, including CD206 and CD209 (27). In that study, the CD14+ dermal APCs were suggested to exhibit a less mature phenotype than CD1a+ APCs, representing a DC subset in the dermis, and potentially a precursor for another mature APC subset in the skin, such as CD1a+ DCs (27). However, in the present study, LP CD14+ Møs did not change their phenotypes into CD1a- DCs even after commensal Ag exposure (data not shown). Additionally, CD14+CD1a- CD209+ cells were previously reported in the skin as an individual DC subset, while this subset exhibited weaker Ag presenting function compared with other DC subsets in the skin, such as CD1a+ DCs (28). However, in that study, the CD14+ dermal DCs produced robust expression of proinflammatory cytokines IL-6, IL-1β, IL-12p40, GM-CSF, and TNF-α, and could induce different types of acquired immune responses compared with other DC subsets. Thus, in the present study, we suggest that the CD14+CD209+ LP APC subsets do not represent a precursor for other mature DC subsets in the intestine, but possess some DC-like Ag presenting functions and may play important roles in gut immunity via these APC functions.

RA signaling has been recently examined as a potential mechanism of immune regulation in the gut. Gut-related tissue-derived APCs highly express the RA synthetic enzyme RALDH, and they can metabolize retinal into all-trans RA (16, 17). RA derived from gut APCs participates in the induction of expression of gut-homing receptors on T and B cells (16, 29), as well as promotion of Foxp3+ Tregs (7, 30-32) and/or IgA+ plasma cells (22, 29). In the present study, the CD14+ LP Møs expressed the RALDH2 and RDH10 genes and strongly induced expression of the gut-homing markers integrin β7 on T cells via self-generation of RA. However, unlike murine LP Møs, the LP CD14+ Møs in humans did not induce IL-10-producing Foxp3+ Treg differentiation even when isolated from normal control mucosa (data not shown). Together with the fact that the LP CD14+ Mø was markedly increased in the inflamed mucosa of IBD patients, particularly in those patients with CD, the LP CD14+ Møs may play pathogenic roles in the intestine via induction of inflammatory Th1 and Th17 subsets.

RA signaling also plays an important role in gut-tropic T cell induction and in regulation of Th17 immunity. RA, which is generated by gut-specific APCs, inhibits the differentiation of Th17 cells but enhances the differentiation of Tregs with TGF-β (18, 33). Indeed, in the present study, a Th17 inhibitory effect by RA was confirmed using either RA supplementation or inhibition of RA signaling with LE540 (Fig. 6 and data not shown). In contrast, the induction of Th17 cells caused by inhibition of RA signaling was not observed when Mo-DCs were used as APCs instead of LP CD14+ Møs (data not shown). These data suggest that RA signaling is a gut-specific regulator of Th17 differentiation, which may play a suppressive role in the Th17-driving by mucosal APCs.

Interestingly, the expression of RDH10 mRNA in LP CD14+ Møs was significantly impaired in patients with CD compared with NC, while there was no difference in RALDH2 expression. RDH10 was reported as an essential enzyme for generation of RA from retinol (34). Hence, in the present study the impaired expression of RDH10 in LP CD14+ Møs in CD patients might lead to insufficient RA signaling. As we have reported the importance of commensal bacteria-induced IL-6 and IL-1β in Th17 differentiation, these data suggest the possibility that increased IL-6 and IL-1β production and impaired RA signaling cooperatively evoked Th17 immunity and subsequent intestinal inflammation in CD patients. However, we also demonstrated that LP CD14+ Møs in CD patients induced gut-homing receptor expression via an RA-dependent manner, although the RA production by LP CD14+ Møs might be impaired in CD patients. Importantly, induction of gut-homing receptors expression with a very low concentration of RA was previously reported (16), while higher concentrations were needed to suppress Th17 differentiation and/or promotion of Treg differentiation (18, 22). Therefore, we think that although the RA producing capacity of LP CD14+ Møs might be impaired in patients with CD, they are able to produce low amounts of RA, which may be enough to induce gut-homing receptors on T cells.

Collectively, the human LP CD14+ Mø subset exhibited DC-like Ag presenting function against naive T cells and promoted predominantly Th1 cells and possibly a small amount of Th17 single- and Th1/Th17 double-positive cells. In the CD patients, potentiated Th1 and Th17 differentiation by LP CD14+ Møs were observed in addition to an increased number of this LP Mø subset. Thus, these observations highlight a role for LP Møs in the enhanced Th1, and potentially in Th17 immunities, and the pathogenesis of intestinal inflammation in CD.

Acknowledgments
We thank Drs. Y. Iwao, H. Ogata, N. Inoue, T. Yajima, and M. Naganuma (Keio University) for helpful discussions and critical comments. We thank H. Naruse, S. Ando, Y. Oshima, K. Arai, and Y. Wada (Keio University) for technical assistance.

Disclosures
The authors have no financial conflicts of interest.

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