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Inherent potential for production of tumor necrosis factor-α by human intestinal macrophages

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Abstract *Background and aims:* Tumor necrosis factor (TNF) production by the macrophages in intestines appears to play a critical role in the pathogenesis of Crohn's disease (CD). However, it is reported that resident intestinal macrophages (both colonic and small-bowel) do not produce TNF after lipopolysaccharide (LPS) stimulation. It has not yet been proven whether or not intestinal macrophages have an inherent potential to produce TNF. The purpose of this study is to answer this question. *Materials and methods:* Colonic macrophages were isolated from lamina propria of human large intestine and stimulated with a variety of substances: LPS, a lipid A derivative (ONO-4007), killed *Streptococcus* bacterial body (OK-432), phorbol 12-myristate 13-acetate, and lectins (pokeweed mitogen and Sarcophaga lectin). *Results:* Colonic macrophages were phenotypically negative for CD14 and positive for CD68 and produced very little TNF in response to LPS, as reported previously. Of the substances tested, only Sarcophaga lectin, which is a defense protein of fleshflies (*Sarcophaga peregrina*), induced TNF production by the intestinal macrophages. In addition, when the colonic macrophages were cultured on immunoglobulin-A-coated dishes, their characteristic response to LPS was altered, and they produced TNF at a level 6.6 times higher than when on collagen-coated dishes. *Conclusion:* Colonic macrophages have an inherent ability to produce TNF. Activation of colonic macrophages by unknown substances may contribute to the induction of TNF production, which causes the intestinal inflammation of CD.

Keywords TNF · LPS · Colonic macrophages · IgA · Crohn's disease

Introduction

Crohn's disease (CD) is a chronic recurrent inflammatory bowel disease of unknown etiology. Several reports suggested that TNF might be a factor that was responsible for pathogenesis of CD. The polymorphism of the promoter region of the TNF gene caused high levels of TNF production and was related to higher risk of CD. Mononuclear cells distributed throughout the interstitium in intestines of CD patients stained prominently for TNF [1]. Commercially available drugs, anti-TNF antibodies, or specific inhibitors of TNF production (thalidomide) have been developed which could inhibit the chronic and acute inflammation of CD [2, 3]. Thus, a critical question is to

determine which cells are responsible for the production of the TNF that causes CD.

Recently, the continuous production of proinflammatory cytokines from intestinal epithelial cells (IEC) has attracted attention to its etiological significance for CD. While a primary culture of IEC did not react with lipopolysaccharide (LPS), they remained sensitive to TNF and produced proinflammatory cytokines [4]. Moreover, IEC acquired the capability of responding to LPS after TNF stimulation through induction of TLR4 on their membranes [5, 6]. TLR4 is a major receptor of LPS from Gram-negative bacteria and transmits the signals for the production of proinflammatory cytokines, including TNF [7]. These results demonstrated that IEC stimulated by TNF may have a prominent role in causing CD, but it is not yet clear whether they are the primary cells responsible for the TNF supply. A clarification of which cells are responsible for producing TNF in a normal intestine may provide useful insight for investigating the mechanism of the etiology of CD.

Macrophages are known as the major TNF producer in vivo and in vitro. The gastrointestinal mucosa is the largest reservoir of tissue macrophages in the body [8]. However, several reports have shown little production of TNF by either colonic or small-bowel macrophages in response to bacterial products such as LPS [9–11]. The mechanism for hyporesponsiveness of intestinal macrophages to LPS is explained in part by their lack of CD14, a GPI-linked glycoprotein that acts as a high-affinity receptor for complexes of LPS and LPS-binding proteins [10, 12–14]. The absence of CD14 on intestinal macrophages markedly suppresses production of proinflammatory cytokines following LPS stimulation [10, 15]. It was reported that the TNF-producing cells in CD lesions were CD14-positive macrophages which were derived from monocytes which had migrated to the inflamed lamina propria [9, 15]. However, it is not yet clear whether the intestinal macrophages are involved as the primary source of TNF.

A few reports have demonstrated a physiological role for TNF in the intestine. Knockout experiments showed that TNF was indispensable in the formation of the second lymphoid organ in intestinal lymph nodes during embryonic development [16]. Also, in an experimental model of colonic *Trichuris muris* infection, the importance of TNF in preventing infection was demonstrated using TNF-receptor-depleted mice [17]. Castagliuolo et al. demonstrated that after injection of toxin A from *Clostridium difficile* into intestinal (ileal) loops in rats, TNF was induced by isolated small-bowel macrophages from the inflamed intestine [18]. These facts suggest that intestinal macrophages have the potential of producing TNF under specific conditions. Thus, we hypothesize that intestinal macrophages can produce TNF when exposed to specific stimuli or under specific circumstances which lead to a change in their characteristics, especially their response to LPS.

This study was performed in order to clarify whether intestinal macrophages possess the ability to produce TNF and especially focused on colonic macrophages, as these were thought to be highly resistant to TNF production in response to LPS. Hyporesponsiveness of colonic macrophages may be essential for avoiding detrimental inflammation from the LPS that is abundant in colonic mucosa. In this study, we isolated normal human colonic macrophages and investigated their TNF production following exposure to various stimuli. The human intestinal macrophages did not produce TNF when stimulated with LPS or other known macrophage stimulators as reported previously [11, 19, 20]. To stimulate colonic macrophages, we chose certain lectins which were known to be phylogenetically conserved defense molecules in various animals and plants. Significant amounts of TNF were induced only after treating with Sarcophaga lectin, a lectin isolated from fly larvae (*Sarcophaga peregrina*) [21, 22]. Moreover, we found that colonic macrophages became capable of production of TNF in response to LPS after contact with IgA.

Patients and methods

Patients

Normal colonic mucosa were obtained from intestines of 30 colorectal cancer patients who underwent surgical resection of the large intestine. Tissues were taken at least 10 cm distant from tumors. The mucosa from each patient was macroscopically normal. The Ethics Committee of Takano Hospital, Kumamoto, Japan, approved these studies, and informed consent was obtained from the patients.

Colonic macrophages

Human colonic macrophages from lamina propria were isolated according to the method described previously [23, 24], with slight modifications. Briefly, stripped mucosa was treated with 1 mM ditiothreitol (Wako Chemicals, Osaka, Japan) to remove mucus and then incubated in 1 mM EDTA (Chemical Dojin, Osaka, Japan) to strip intestinal epithelial cells from the intestine. Then tissues were minced and incubated for 60 min in 500 U/ml collagenase (Wako Chemicals) and 1,000 U/ml dispase (Godou Syusei, Tokyo, Japan). The digest was filtered by stainless mesh and then centrifuged through Ficoll–Paque (Amersham Pharmacia Biotech, Tokyo, Japan). The resultant preparation of lamina propria mononuclear cells (LPMNCs) that were collected from the Ficoll–medium interface was poured into dishes coated with collagen (300 μg/ml, cell matrix type I-C, Nitta Gelatin, Osaka, Japan). After incubation overnight at 37°C in 5% CO_2, the nonadherent cells were removed with warm PBS, and adherent cells were subjected to analyses of surface phenotype or TNF production.

The adherent cells were incubated in 10 mM EDTA in PBS and harvested with a cell scraper and then counted. They were then analyzed by flow cytometry (EPICS XL, Coulter, FL, USA). Then the cells were treated with anti-CD14 conjugated with fluorescein isothiocyanate (FITC, Becton-Dickinson, Bedford, MA, USA), anti-CD89 conjugated with phycoerythrin (PE), anti-CD3-PE, and anti-CD19-PE or isotype of control antibodies (Dako, Kyoto, Japan). To examine the surface expression of CD68, we used anti-CD68 (Dako) that was not conjugated with fluorescent reagent, so we incubated anti-CD68 treated cells with rabbit antimouse immunoglobulin conjugated with FITC.

To prepare immunoglobulin-coated dishes, γ-globulin (300 μg/ml) (Wako Chemicals), human IgG (200 μg/ml), and human IgA (200 μg/ml) were poured into separate plastic dishes and left for 20 min. Then plates were washed with PBS. Normal human IgG and IgA were purified from normal human plasma according to the methods described previously [25, 26]. LPMNCs of 6×10^6 cells/ml were poured into dishes coated with each immunoglobulin, were kept overnight, and were then washed with warm PBS.

Isolation of human peripheral blood monocytes

Peripheral blood was drawn with heparinized test tubes from five healthy donors and two patients who were about to undergo operation. The blood was centrifuged over a Ficoll–Hypaque (Amersham Pharmacia Biotech) layer. The blood mononuclear cells after Ficoll isolation were resuspended in the medium, and peripheral blood monocytes (PBM) were purified in the dishes coated with the proteins as described above.

TNF production

Isolated adherent cells ($0.4–1\times10^5$ cells/well) obtained by the method described in the previous section were treated with various macrophage-activating agents. The supernatants were collected over time to analyze TNF activity. The agents used were as follows: *Escherichia coli* LPS (O127: B8, 10 μg/ml; Difco, Detroit, MI, USA); phorbol 12-myristate 13-acetate (PMA, 100 ng/ml; Sigma, St. Louis, MO, USA); lipoteichoic acid (LTA, 100 μg/ml; Sigma); a lipid A derivative (ONO-4007 [27]; provided by ONO Pharmaceutical Co., Osaka, Japan); OK-432 [20, 28] (1 KE/ml; 0.1 mg dry body weight, provided by Chugai Pharmaceutical Co., Tokyo, Japan); PSK [28] (100 μg/ml; provided by Kureha Chemical Industry Co., Tokyo, Japan); and pokeweed mitogen (PWM, 10 μg/ml; Sigma). Sarcophaga lectin was purified in our laboratory [21, 22]. TNF activity was measured by L-929 cytotoxic assay as previously described [29]. The detection limit of TNF activity was less than 0.1 $U/10^5$ cells.

Statistical analysis

Statistical evaluations of differences between groups were made by Student's *t* test, paired *t* test, or Mann–Whitney *U* test.

Results

Colonic macrophages did not respond to LPS

A typical characteristic of human intestinal macrophages is that they do not produce TNF in response to LPS. This is associated with their near lack of CD14 expression on their cell surfaces [9–13]. To confirm these observations, colonic macrophages were collected and tested as described in the previous section.

The phenotype of colonic macrophages expressed $CD68^+$, $CD14^-$, $CD19^-$, and $CD3^-$, and PBM expressed $CD68^+$, $CD14^+$, $CD19^-$, and $CD3^-$. Phenotypes of the collected colonic macrophages and PBM are summarized in Table 1. The supernatants of the colonic macrophages ($0.4–1\times10^5$ cells/well) were collected over time after addition of LPS (concentration 10 μg/ml), and TNF activity was then measured.

As shown in Fig. 1, 3 h after treatment with LPS, PBM produced significant amounts of TNF at a concentration of 13.9±6.2 $U/10^5$ cells as measured by the L-929 cytotoxic assay. Compared to PBM, only 3.5% of TNF (0.5±0.9 $U/10^5$ cells) was produced by colonic macrophages. Moreover, the amount of TNF produced by colonic macrophages 3 h after addition of LPS was not statistically significantly different than the control (0 h after addition of LPS). These results are consistent with previously reported results [30].

TNF production by colonic macrophages in response to macrophage-activating agents

To investigate TNF production by colonic macrophages, the cells were exposed to a number of macrophage-activating agents. These substances and the reasons for their selection are as follows: (1) *E. coli* LPS is effective in inducing TNF production mainly via the toll-like receptor 4 (TLR4); (2) it is well known that PMA induces TNF by a different mechanism than LPS [31]; (3) LTA is a membrane component of Gram-positive bacteria that has the potential

Table 1 Comparison of colonic macrophage and PBM phenotypes

Cell	CD68	CD14	CD19	CD3
PBM	79±16%	92±5.3%	3.0±1.3%	2.8±1.3%
Colonic macrophages	77±18%	0.9±0.4%	2.3±0.6%	6.9±1.6%

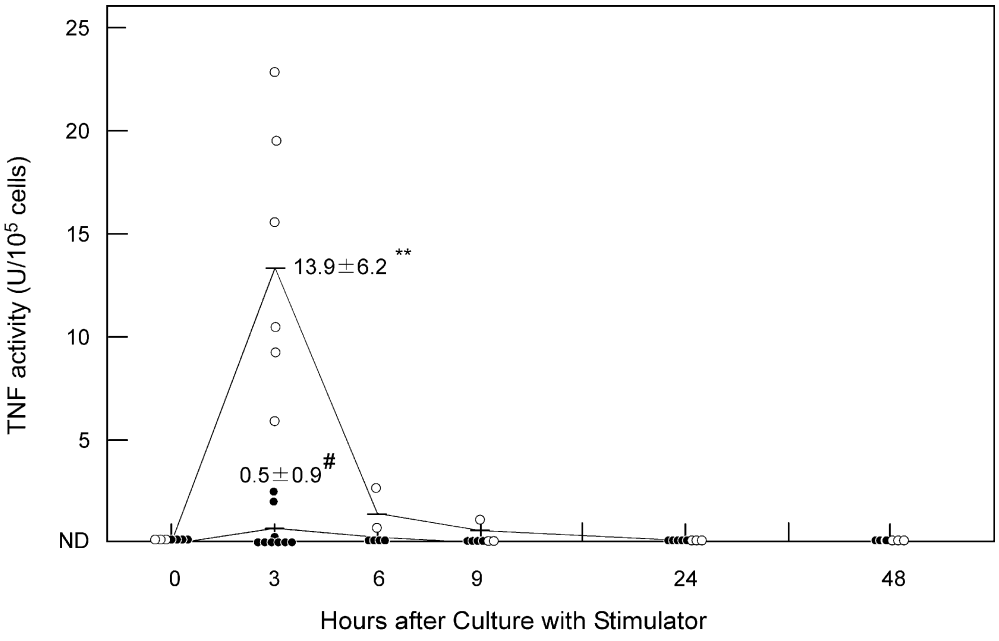

Fig. 1 Production of TNF by PBM and colonic macrophages after addition of LPS. Isolated PBM or colonic macrophages ($0.4–1\times10^5$ cells/well) were treated with LPS (10 μg/ml); then supernatants were collected with time. TNF activity was measured by L-929 cytotoxic assay. TNF activity was calculated for 1×10^5 cells. *Open circles* PBM (n=6) treated with LPS, *closed circles* colonic macrophages (n=12) with LPS. *Bars* indicate mean values of individual samples. ** Significant difference (P<0.001, Student's t test and Mann–Whitney U test) from colonic macrophages group, # no significant difference (Student's t test and Mann–Whitney U test) from 0-h group (without stimulation)

to induce TNF mainly via TLR2 [32]; (4) ONO-4007 is known to induce TNF via a non-CD14 cascade [27]; (5) OK-432, a commercially available *Streptococcus* bacterial body, is reported to induce TNF in vivo and in vitro [20, 28]; and (6) PSK is a commercially available antitumor polysaccharide that is known to activate macrophages when administered orally [28].

As shown in Fig. 2, PMA, ONO-4007, and PSK did not induce TNF by colonic macrophages, and LPS and LTA induced 0.5 and 0.9 $U/10^5$ cells of TNF, respectively. This is only 5% of the amount of TNF that was produced by PBM.

TNF production by colonic macrophages in response to plant and animal lectins

Previous reports suggested that human isolated LPMNCs which contained T cells, B cells, and macrophages could produce TNF in response to PWM [13]. This activity is more than ten times higher than that produced with LPS. Thus, PWM was investigated to determine if it would induce TNF production by colonic macrophages.

As shown in Fig. 2, PBM and colonic macrophages produced 2.7±1.2 and 0.6±1.0 $U/10^5$ cells of TNF, respectively. The amount of TNF produced by colonic macro-

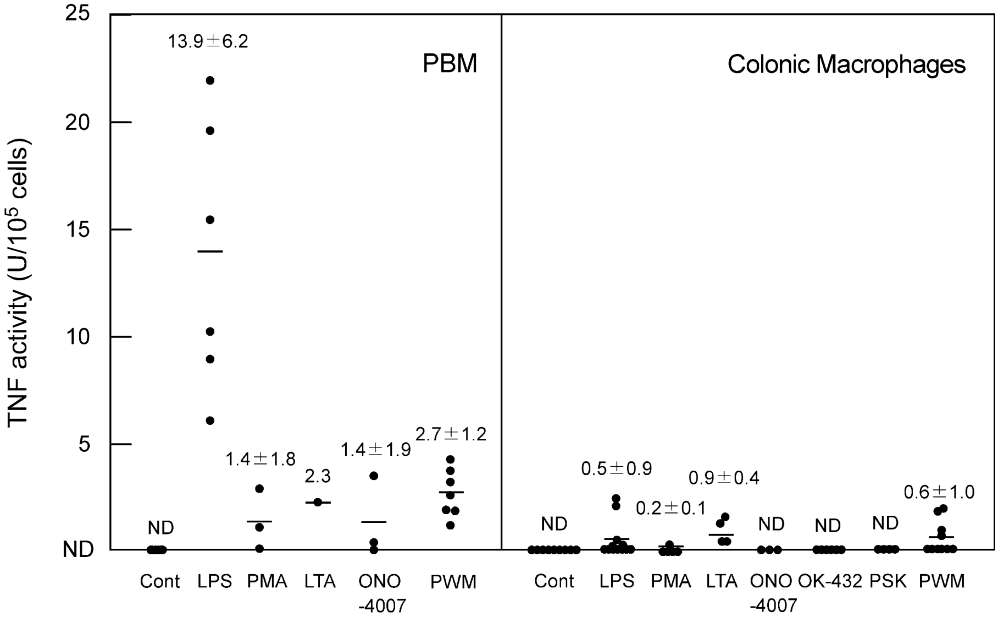

Fig. 2 Production of TNF by PBM and colonic macrophages treated with various macrophage-activating agents. Isolated PBM or colonic macrophages were treated with various agents; 3 h later, supernatants were collected. TNF activity was measured by L-929 cytotoxic assay. Because there was dispersion of the cell number ($0.4–1\times10^5$ cells/well), TNF activity was calculated for 1×10^5 cells. Each *dot* and *bar* indicates the individual value and average. *ND* Not detectable (less than 0.1 $U/10^5$ cells), *Cont* control (without stimulation)

Table 2a Production of TNF by human colonic macrophages when treated with Sarcophaga lectin

Stimulator	Dose (μg/ml)	TNF activity (U/10^5 cells)		
		3 h	6 h	24 h
None	–	ND	ND	ND
LPS	10	ND	0.7	0.3
PWM	10	ND	0.5	ND
Sarcophaga lectin	0.1	0.2	0.5	ND
	1	0.7	2.6	ND
	10	0.6	1.6	ND

Colonic macrophages (0.4–1×10^5 cells/well) were treated with LPS, PWM, or Sarcophaga lectin. Supernatants were collected at 3, 6, and 24 h
ND Not detectable (less than 0.1 U/10^5 cells)

phages is not statistically significant when compared to that produced with LPS. These results suggested that the major cells responsible for TNF production by PWM in a previous report [13] might be attributable to nonadherent cells.

We then tested whether an animal lectin (Sarcophaga lectin) induced TNF production. Sarcophaga lectin is 190 kDa of *S. peregrina* C-type lectin, and galactose is the inhibitory sugar which binds it [33]. Moreover, Sarcophaga lectin from *S. peregrina* is known as a biological defense factor and is reported to induce TNF production in a mouse macrophage cell line (J774.1) [34].

Sarcophaga lectin (100 ng/ml to 10 μg/ml) induced significant amounts of TNF by human colonic macrophages as shown in Table 2a, which was 3.7 times higher than when LPS (10 μg/ml) was used. Mouse colonic macrophages were tested to confirm production of TNF in response to Sarcophaga lectin.

At time intervals of 3, 6, and 24 h after stimulation, 0.4 to 2.1 U/ml of TNF activity was induced in response to Sarcophaga lectin (0.1 to 10 μg/ml) (Table 2b). Antimouse TNF antiserum showed complete inhibition of cytotoxic activity by murine colonic macrophages treated with Sarcophaga lectin (Table 2b). The amount of TNF produced by colonic macrophages in response to Sarcophaga lectin was not higher than that produced by PBM in response to LPS. However, the amount of TNF produced by Sarcophaga lectin was statistically significantly different when compared to the amount of TNF without Sarcophaga lectin. These results demonstrated that colonic macrophages inherently possess the capability to produce TNF in response to certain stimuli.

TNF production by colonic macrophages followed by culture on immunoglobulin-coated dishes

We examined TNF production by colonic macrophages in response to natural substances that the macrophages might encounter under normal physiological conditions. We focused our attention on an antibody, specifically IgA, for the following reasons. It has been shown that TNF production by a variety of macrophages in response to LPS was augmented if the macrophages were pretreated with immunoglobulin-coated glass fibers [35] or erythrocytes [36]. Moreover, the intestine is believed to be a tissue where abundant antibodies, especially IgA, are produced. IgA is one of the major constituents used by the mucosal barrier for protection against microbial infections.

We investigated TNF production by colonic macrophages through cultivation on immunoglobulin-coated dishes. LPMNCs were cultured overnight on dishes coated with collagen (control), γ-globulin, IgG, or IgA. Nonadherent cells were then washed with PBS, and remaining adherent colonic macrophages were stimulated with LPS. Maxell et al. reported that colonic macrophages cultured on γ-globulin-coated dishes had the same phenotype as reported previously (CD68-positive and CD14-negative). We also checked CD68-positive and CD89-negative phenotypes of the colonic macrophages used (data not shown).

As shown in Fig. 3, colonic macrophages cultured on γ-globulin-, IgG-, or IgA-coated dishes produced statistically significant amounts of TNF in response to LPS, while there was no statistical significance in the amount of TNF produced by colonic macrophages with or without LPS on the collagen-coated dishes. Colonic macrophages cultured on IgA-coated dishes showed the highest amount of TNF production in response to LPS. The amount of TNF produced by PBM that were cultured on γ-globulin- and IgG-

Table 2b Production of TNF by murine colonic macrophages treated with Sarcophaga lectin

Stimulator	Dose (μg/ml)	TNF activity (U/10^5 cells)					
		3 h	Antibody[a]	6 h	Antibody	24 h	Antibody
None	–	ND	ND	ND	ND	ND	ND
Sarcophaga lectin	0.1	0.5	ND	1.4	ND	0.4	ND
	1	1.9	ND	2.1	ND	1.9	ND
	10	0.4	ND	0.9	ND	ND	ND

Colonic macrophages (0.4–1×10^5 cells/well) were treated with Sarcophaga lectin. Supernatants were collected at 3, 6, and 24 h
ND Not detectable (less than 0.1 U/10^5 cells)
[a]TNF assay with antimouse TNF antiserum

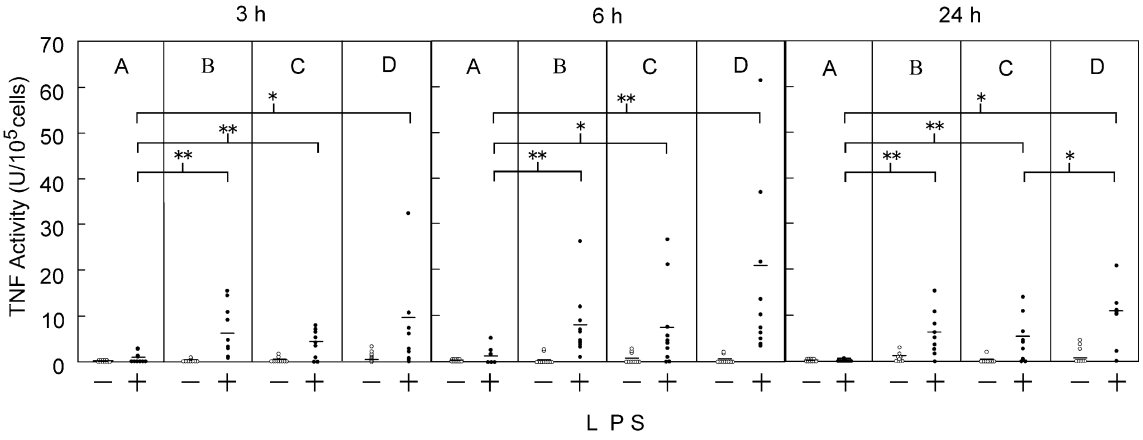

Fig. 3 Production of TNF by colonic macrophages following culture on immunoglobulin-coated dishes. LPMNCs were poured into dishes coated with one of the following substances: *A* collagen (300 μg/ml), *B* γ-globulin (300 μg/ml), *C* human IgG (200 μg/ml), or *D* human IgA (200 μg/ml). After overnight incubation at 37°C in 5% CO_2, the nonadherent cells were removed with warm PBS. The adherent cells (0.4–1×10^5 cells/well) were treated with LPS (10 μg/ml). Supernatants from wells were collected over time. TNF activity was measured by an L-929 cytotoxic assay. TNF activity was calculated for 1×10^5 cells. Each *dot* and *bar* indicates the individual value (colonic macrophages, n=11) and average. + Stimulation with LPS, − without stimulation, *ND* not detectable (less than 0.1 U/10^5 cells). Statistical analysis was performed by paired t test. *<0.05, **P<0.01

coated dishes was also higher than that by PBM which were cultured on collagen-coated dishes.

Interestingly, enhancement of TNF production by colonic macrophages especially cultured on IgA-coated dishes was different from production by PBM on IgA-coated dishes. As shown in Table 3, regardless of the treatment time with LPS (3, 6, and 24 h), the degree of enhancement of TNF production by colonic macrophages was significantly higher than PBM (6.7 times higher at 3 h, 6.1 times at 6 h, and 4.9 times at 24 h). Thus, colonic macrophages have a different response to IgA-coated dishes than does PBM. These results indicated that colonic macrophages have the potential to produce TNF.

Discussion

Human colonic macrophages were studied to determine if they are inherently capable of producing TNF. As reported previously, isolated human colonic and small-bowel macrophages lacked CD14 surface markers, had a CD68-positive phenotype, and did not produce TNF in response

Table 3 Enhancement of TNF production by colonic macrophages cultured on IgA-coated dish

Stimulator	TNF activity (U/10^5 cells)					
	3 h		6 h		24 h	
	PBM	Colonic macrophages	PBM	Colonic macrophages	PBM	Colonic macrophages
Collagen	47±47[b]	1.2±1.1	42±26	1.6±1.3	14±6.7	0.5±0.5
γ-Globulin	162±103	5.4±4.8	193±111	7.7±7.3	196±106	6.2±5.0
IgG	108±40	4.8±3.1	142±80	7.6±9.2	102±103	4.4±4.6
IgA	41±31	7.2±9.1	75±27	17±19	62±49	10±6.3
Ratio[a]	0.9±0.6	6.0±7.5[c]	1.8±0.7	11±12[d]	4.5±3.5	22±14[e]

LPMNCs or blood mononuclear cells were poured into dishes coated with one of the following substances: collagen (300 μg/ml), γ-globulin (300 μg/ml), human IgG (200 μg/ml), or human IgA (200 μg/ml). After overnight incubation at 37°C in 5% CO_2, the nonadherent cells were removed with warm PBS. The adherent cells (0.4–1×10^5 cells/well) were treated with LPS (10 μg/ml); the supernatants were collected at 3, 6, and 24 h. Statistical analyses were performed by Student's t test and Mann–Whitney U test between relative TNF values of PBM and colonic macrophages

[a]The amount of TNF produced with LPS cultured on IgA-coated dishes divided by production on collagen-coated dishes

[b]Absolute TNF activity (U/10^5 cells) with standard deviations

[c]P=0.128 (t test), P=0.013 (U test)

[d]P=0.088 (t test), P=0.0056 (U test)

[e]P=0.011 (t test), P=0.0389 (U test)

to LPS. However, we found that colonic macrophages did produce TNF in response to Sarcophaga lectin. Moreover, they responded to LPS after contact with IgA. This is the first report indicating that colonic macrophages possess an inherent capability of producing TNF in response to some external as well as physiological stimuli.

In this study, we isolated colonic macrophages by the methods described by Bull and Bookman [24], with slight modifications. Although the elutriation technique provides cells of superior purity (about 99% of collected cells) [12], the method in this study appears to have been adequate because the prepared colonic macrophages showed no TNF production with LPS stimulation. Besides, these cells were phenotypically about 80% positive for CD68 (Table 1). The purity of isolated macrophages in our experiments was estimated as not less than 90% for the following reasons: Colonic macrophages have been reported to be phenotypically only 83% positive for CD33, which is a surface marker of intestinal macrophages coexpressed with CD68. This indicates that the purity of colonic macrophages used in these experiments is probably over 90%. This was sufficient purity for analysis of the specific characteristics in these cells [14]. According to a report by Golder and Doe [23], who collected colonic macrophages by the same method we followed, the purity of colonic macrophages from human intestine was over 90% (phagocytic cell, nonspecific esterase). Thus, the purity of colonic macrophages used in our experiments was equivalent to the preparations reported previously [24].

These contaminant cells probably produced very little TNF because, after removing adherent cells, TNF production by LPMNCs was not observed (data not shown). The ratio of the CD14-positive cells in our preparation was only 0.9±0.4%. These cells were probably derived from monocytes in the blood that contaminated the surgically resected tissue. It is clear that the trivial amount of mixed monocytes from the peripheral blood could not affect the conclusions of this paper, especially the response of colonic macrophages cells to Sarcophaga lectin in Tables 2a and 2b and IgA-coated dishes in Fig. 3 and Table 3.

Although it was thought that normal intestinal macrophages did not produce TNF [11, 19, 20], we hypothesized that intestinal macrophages might be producing TNF for the following reasons: (1) Pleiotropic effects of TNF such as apoptosis, differentiation, and proliferation have already been reported. (2) As part of the primary defense against viruses and bacteria, TNF is produced by macrophages after activation of NF-κB through various pathogen-associated molecular patterns and adaptor molecules such as MyD88 and TRAF. (3) During embryonic development of mammals, TNF is detected throughout most stages of ontogeny starting from the blastocyst stage [37, 38]. (4) In regulating the physiology of the gastrointestinal tract, apparently, TNF is an essential molecule for organogenesis of the second lymphoid organ of the intestine during embryonic development [16]. Each of these observations about lamina propria indicated that TNF is part of the mechanism required to maintain the physiological state in the gastrointestinal tract with or without inflammation.

In order to investigate whether colonic macrophages are inherently capable of producing TNF, we exposed them to several substances that have been reported to possess the ability to activate macrophages and initiate TNF production. However, these substances, by themselves, apparently did not induce colonic macrophages to produce TNF as reported previously [11, 19, 20]. We found that Sarcophaga lectin induced significant production of TNF by colonic macrophages (Tables 2a, 2b). Induced activity was four times higher than with LPS (10 μg/ml).

It was observed that TNF production by the colonic macrophages with Sarcophaga lectin had a bell-shaped pattern (Table 2a, 2b). The same phenomena was observed with induction of macrophage chemotaxis by endothelin-2 [39], neutrophil chemotaxis by macrophage inflammatory protein-2 [40], thymocyte proliferation by IL-1 [41], and T-cell chemotaxis by RANTES [42]. These biological responses are linked to dimer formation of their receptors with self-antagonism at high ligand concentrations, when monomeric receptor complexes become predominant [43].

Our results also showed that colonic macrophages could be activated so that they were capable of producing TNF after contact with immunoglobulin, especially with IgA (Fig. 3 and Table 3). IgA is locally synthesized in and secreted throughout the mucosal surface and has a protective role against the microbes that are encountered on the mucosal surfaces [44, 45]. While almost all IgA molecules are secreted from the intestine, it is known that membrane-bound IgA exists on the membrane surface of B cells which have moved to the intestinal mucosa after receiving antigen sensitization in a Peyer's patch [46, 47]. IgA-expressing cells may come in contact with macrophages that have IgA receptors. This suggests that intestinal macrophages may be activated in this way by the membrane-bound IgA molecules on B cells, and IgA-coated dishes seem to mimic membrane-bound IgA. Thus, a physiological role of membrane-bound IgA might be regulation of TNF production by colonic macrophages, in addition to its protective role against mucosal invasion by microbes. These results suggest that colonic macrophages have IgA receptors on their surface. This is supported by the finding that Fc receptors for IgA (FcαR) were found on phagocytes residing on mucosal surfaces [45]. However, it has been reported that expressions of surface FcαR (CD89) and FcγR were scarce on the surface of intestinal macrophages [10, 11]. While human small-bowel macrophages do not present FcαR, they have another immunoglobulin receptor, a neonatal immunoglobulin receptor (FcRn) which binds to IgG, IgA, and IgM [48]. Moreover, it has recently been reported that there is a polymeric immunoglobulin receptor, FcαμR, that is able to bind to IgM and IgA and that is expressed on the majority of B lymphocytes and macrophages [49].

Although, the functions of the receptors that bind to immunoglobulins have not yet been clarified, these facts suggest that intestinal macrophages may provoke an immune cascade after binding with IgA-antigen complexes to its receptors.

The physiological significance of the TNF produced by intestinal macrophages is not yet clear. We postulate that occasional TNF production by intestinal macrophages might occur in a limited region of the mucosal surface, and this could play an important role in host defenses against luminal microorganisms.

The etiology of CD is not yet clear. In this paper, we clarified that TNF could be produced by resident colonic macrophages. Thus, colonic macrophages seem to be involved in the etiology of CD. This research suggests that a more complete understanding of resident intestinal macrophages would aid in resolving the mechanism of the etiology of CD.

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