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Review

Macrophages in Muscle Layer of Gastrointestinal Tract: Impairment of Muscle Contraction by Treatment with Lipopolysaccharide

Shigeko Torihashi[1], Masatoshi Hori[2] and Hiroshi Ozaki[2]

[1]Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, Tsurumai, Nagoya 466–8550 and [2]Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–8657

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The morphology and function of resident macrophages in the muscle layer and in the subserosa of the gastrointestinal tract are described. FITC-dextran injected via the tail vein of a mouse or rat demonstrated a regular arrangement of macrophages on a whole mount preparation of the muscle layer. Electron microscopy revealed inactive phagocytosis under normal conditions. After a 4–8 hr incubation of rat ileal muscle with lipopolysaccharide (LPS) to investigate its effect on muscle contraction, macrophages began to express inducible nitric oxide synthase (iNOS) and released nitric oxide. This macrophage response was accompanied by the expression of cyclooxygenase-2 (COX-2) immunoreactivity and the production of prostaglandins. The muscarinic agonist-induced contraction was greatly inhibited by LPS treatment, and this inhibition was reversed by either iNOS or COX-2 inhibitors. We concluded that one of the functions of muscular macrophages was the modulation of smooth muscle contraction under pathological conditions. The mechanism of iNOS upregulation indicated by our experiment is discussed.

Key words: Macrophage, Gastrointestinal tract, Smooth muscle, Inducible nitric oxide synthase, Cyclooxygenase-2

I. Introduction

Recent investigations have revealed that a great number of macrophages are distributed in the muscle layer and in the subserosa of the gastrointestinal (GI) tract of mammals including humans [14–18]. They are called muscular macrophages and are categorized as a resident type [22]. In general the most common function of macrophages is phagocytosis and the presentation of digested antigens at their cell surface to induce immune responses and to activate other migratory immune cells [12, 20, 21]. Another important function is cytokine release to activate the immune response and alter the biological activities of surrounding cells. Accordingly, if muscular macrophages release active substances in the muscle layer, they may affect smooth muscles and enteric neurons nearby. Although little is know about the characteristics and functions of muscle macrophages, it has been strongly suggested that they affect smooth muscle contraction and modulate GI motility under pathological conditions [4, 6, 8, 19]. Recent reports have revealed that inflammatory diseases of the GI tract such as Crohn's disease suppress GI motility [1, 2]. In this review, we summarize their affect on smooth muscles and the modulation of GI motility from our current investigations.

Correspondence to: Shigeko Torihashi, Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466–8550, Japan.

II. Morphology of Muscular Macrophages

Macrophages are distributed in the muscle layer in a distinct manner [14–18]. In small rodents such as mice, rats and guinea-pigs, macrophages are distributed almost evenly

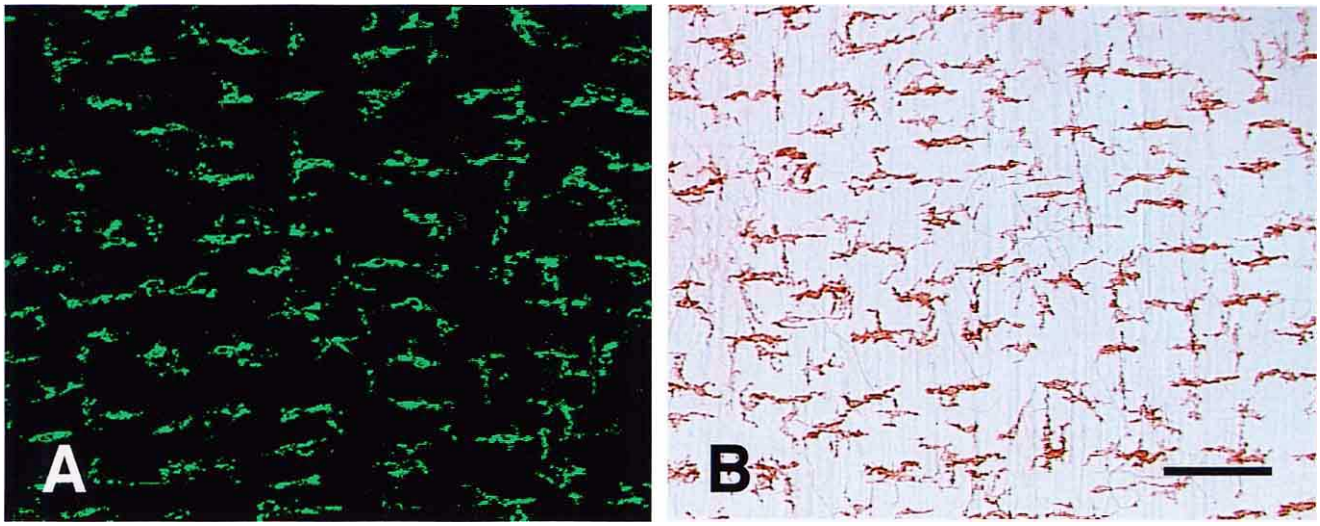

Fig. 1. Whole mount preparation showing resident macrophages in the muscle layer and subserosa of the rat ileum. **A:** Macrophages were illuminated with FITC after 2 days injection of FITC-dextran via tail vein. They are located almost evenly. **B:** Immunohistochemistry of a marker for resident macrophages (clone ED2). A typical appearance of macrophages is a multipolar cell with long cell processes protruding spikes. Almost FITC-dextran uptaking cells expressed immunoreactivity for ED2 [22]. Bar=100 μm (**A** and **B**).

in the subserosa, at levels of the myenteric plexus throughout the GI tract, and at the deep muscular plexus in the small bowel. This distribution pattern is not obvious in the human tissue in which macrophages are scattered among smooth muscle cells of both circular and longitudinal muscle layers [15]. When FITC-dextran was injected via the tail vein of a mouse or rat, macrophages were illuminated with the uptake of FITC-dextran. Whole mount preparations of the muscle layer after injection of FITC-dextran clearly demonstrated their three-dimensional distribution (Fig. 1A).

Electron microscopy demonstrated typical ultrastructural features of macrophages such as dense bodies indicating lysosomes, coated vesicles, and heterogeneous vesicles suggesting phagosomes. Compared to mucosal macrophages their phagosomes were not prominent in the intact tissue indicating inactive phagocytosis [14, 15, 22].

Several markers are useful for staining macrophages by immunohistochemistry, though immunoreactivities differed according to the animal species. Almost all muscular macrophages in rats were immunopositive for a marker of resident macrophage (clone ED2 in Fig. 1B). However, a few of them expressed MHC class II (clone OX6) [22]. Mouse macrophages usually expressed MHC class II and a maturation marker for mononuclear phagocytes (F4/80 antigen) [18].

III. Inhibition of Muscle Contraction by Lipopolysaccharide (LPS) Treatment

Recently, Eskandari *et al.* [3] reported that an intraperitoneal injection of endotoxine lipopolysaccharide (LPS) activated muscular macrophages and suppressed circular muscle contraction in rats. From this description we came up with an experiment to incubate muscle layers with LPS (100 μg/ml) in which the possible contribution of other immune cells that migrated from the blood stream could be excluded. After a 4–8 hr incubation, circular muscle contraction was measured, and then samples were stained with anti-inducible nitric oxide synthase (iNOS) antibody as a whole mount preparation to know whether macrophages induce this enzyme after LPS treatment [22]. Rat muscle layers from an ileal segment (2–3 cm long) where macrophages did not usually express iNOS immunoreactivity, were used. After treatment with LPS, smooth muscle contractility was reduced compared to control tissue incubated without LPS, and this reduction was recovered by addition of iNOS blocker to the incubation medium containing LPS (Fig. 2). Immunohistochemistry revealed that macrophages began to express iNOS, and that the number of iNOS-expressing cells increased in a time-dependent manner, although the total number of macrophages did not alter during incubation. It was confirmed by double labeling that iNOS immunopositive cells showed immunoreactivity for anti-macrophage antibody ED2 [7]. The measurement of iNOS mRNA by RT-PCR also supported an increase in iNOS. Moreover, the amount of NO released into the incubation medium rose during LPS treatment [7]. These findings indicate that muscular macrophages were activated by LPS treatment and induced iNOS producing NO to inhibit smooth muscle contraction. Thus we concluded that one of the functions of muscular macrophages was the modulation of GI motility as an inflammatory response *in vivo*.

IV. Mechanism of iNOS Upregulation

LPS treatment increased the population of muscular macrophages expressing cyclooxygenase-2 (COX-2) immunoreactivity as well as iNOS. COX-2 is an inducible enzyme

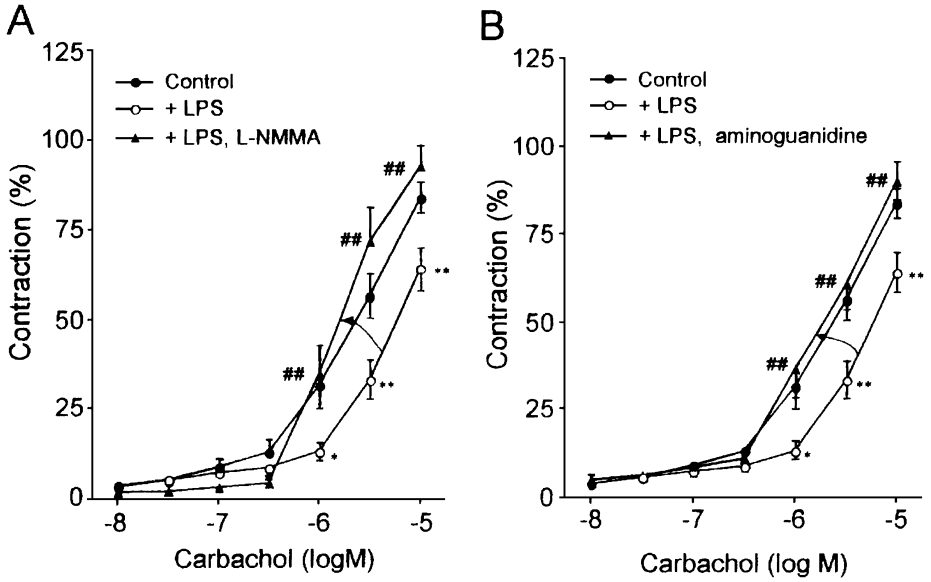

Fig. 2. Inhibition of mechanical contraction by LPS treatment and its recovery by iNOS inhibitors in rat ileac circular smooth muscles. Samples were incubated with or without LPS (100 μg/ml) for 4 hr before measuring contractions. After the treatment with LPS, carbachol (10^{-8}–10^{-5} M) was cumulatively added to the ileac strip to induce contractions. Samples were also incubated with L-NMMA (300 μM; **A**) or aminoguanidine (300 μM; **B**) for 4 hr. Values are expressed as percentage of the reference response (72.2 mM KCl) in fresh tissues. Results are indicated as the mean±S.E.M. (n=9–11) *P<0.05 or **P<0.01 vs. control. ##P<0.01 vs. (+) LPS. This figure was redrawn, with permission, from ref. [22].

like iNOS and produces prostaglandins (PGs). Increases in COX-2 and PGs by treatment with LPS were confirmed by the raise in COX-2 mRNA and both prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) levels measured by the enzyme-immunoassay system [7]. Inflammation mediator PGs and NO have the ability to directly affect GI smooth muscle. However, their crosstalk has been suggested. The interaction between NO and PGs is complicated and differs in various cell types [5]. Since muscular macrophages in rat ileum express both iNOS and COX-2 and release NO and PGs after LPS treatment, it was proper to consider the interaction between them. Our experiments led to some hypothesis of how these inflammation mediators work and interact with each other in the muscular macrophage [7].

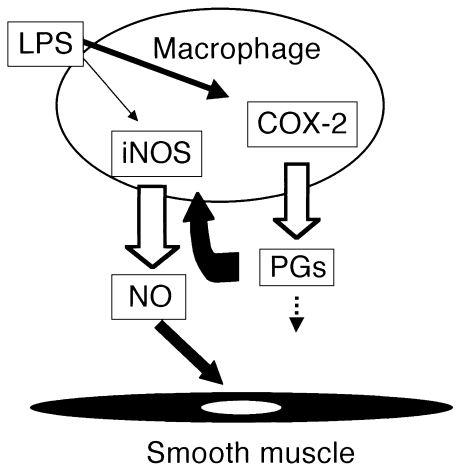

Fig. 3. Schematic view of the relation between iNOS and COX-2 after LPS treatment. LPS induced primarily COX-2 in the resident muscular macrophage to produce PGs, then enhanced iNOS gene expression which may be mediated by PGs. The combined reactions resulted in NO release from resident macrophages to inhibit smooth muscle contraction.

The time course of iNOS and COX-2 mRNA expression during incubation with LPS revealed that COX-2 expression was prior to iNOS expression. During the first 15 min of incubation, mRNA of COX-2 began to increase, while that of iNOS occurred after 90 min. Induction of iNOS indicated by the increase in mRNA was inhibited by the addition of COX-2 inhibitor but not by the addition of iNOS inhibitor during incubation with LPS. On the other hand, COX-2 induction was not suppressed by either COX-2 or iNOS inhibitors. The mechanical contraction of the muscle was reduced by treatment with LPS as mentioned above. This reduction was recovered by the addition of either iNOS inhibitor or COX-2 inhibitor [7]. Therefore, the reduction in muscle contractility was caused mainly by NO, and the prostaglandin produced by COX-2 was involved in the production of NO to inhibit contraction indirectly. The suggested mechanism of circular muscle relaxation by LPS treatment is summarized in Fig. 3. LPS induced primarily COX-2 in the muscular macrophage to produce PGs. It stimulated iNOS expression probably in an autocrine manner. Macrophages then released NO to inhibit smooth muscle mechanical activity.

V. Conclusion and Perspectives

We demonstrate here that muscular macrophages modulates circular muscle contraction under endotoxin LPS treatment through the upregulation of COX-2 and iNOS in rat muscular macrophages. In this experiment we also found that macrophages expressing major histocompatibility complex (MHC) class II increased in number. Thus, it is possible that macrophages emphasize antigen presentation and enhance immune responses in the muscle layer. Involvement of intestinal dendritic cells for the immune response also cannot be over looked [13]. Macrophages may mobilize and activate other immune cells to release cytokines *in vivo*. Recently, Lodato *et al.* [11] demonstrated that LPS treatment impairs longitudinal smooth muscle contraction in the rat ileum. Moreover, the same authors found that iNOS, interleukin-1 and tumor necrosis factor-α are released and mediate the decrease in muscle contraction. Although, the sources of these cytokines were not indicated, it is strongly suggested that several immune cells are involved. Kalff *et al.* described that leukocytes migrating into the muscle layer released inflammatory mediators and initiated muscle dysfunction as an inflammatory response [9, 10]. Thus muscular macrophages have the potential to modulate muscle contraction through releasing biologically active substances directly and through activating and mobilizing other immune cells under pathological conditions. Further investigation of macrophage behavior in the muscle layer may help to better understand the dysfunction of GI muscle during infection or inflammatory disease.

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VII. References

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