Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-κB transcription factors

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Erythromycin and other macrolides are effective for the treatment of chronic inflammatory airway diseases such as diffuse panbronchiolitis (DPB) and chronic sinusitis. The effect of macrolides in DPB is suggested to be anti-inflammatory rather than antibacterial. We investigated the effects of clarithromycin on interleukin-8 (IL-8) production using human peripheral monocytes and the human monocytic leukaemia cell line, THP-1. Bacterial extracts from Escherichia coli, Pseudomonas aeruginosa and Helicobacter pylori, as well as E. coli-derived lipopolysaccharide (LPS), induced IL-8 production. Clarithromycin suppressed this production in a dose-dependent manner in both monocytes and THP-1 cells (49.3–75.0% inhibition at 10 mg/L). A luciferase reporter gene assay with plasmids containing a serially deleted IL-8 promoter fragment showed that both the activator protein-1 (AP-1) and/or the nuclear factor-κB (NF-κB) binding sequences were responsible for the LPS and clarithromycin responsiveness of the IL-8 promoter. Consistently, in an electromobility shift assay, LPS increased the specific binding of both AP-1 and NF-κB, whereas clarithromycin suppressed it. Moreover, LPS and clarithromycin regulated three other promoters that have either the NF-κB or the AP-1 binding sequences: two synthetic (pAP-1-Luc and pNF-κB-Luc) and one naturally occurring (ELAM-Luc). Our results indicate that clarithromycin modified inflammation by suppressing IL-8 production and that clarithromycin may affect the expression of other genes through AP-1 and NF-κB. In addition to treatment of airway diseases, the anti-inflammatory effect of macrolides may be beneficial for the treatment of other inflammatory diseases such as chronic gastritis caused by H. pylori.

Introduction

Macrolides with a 14-member ring, such as erythromycin or clarithromycin, are bifunctional drugs. They have an anti-inflammatory effect in addition to their antimicrobial effect.1–8 The establishment of long-term macrolide therapy as an effective regimen for diffuse panbronchiolitis (DPB) first brought to light their anti-inflammatory effect.9,10 DPB is characterized by chronic sinopulmonary infection and inflammation that is resistant to the usual antibiotic or corticosteroid therapies. In the early phase of the disease, Haemophilus influenzae is the primary infecting microbe. After repeated cycles of exacerbation and remission, Pseudomonas aeruginosa becomes the predominant organism and patients die of chronic respiratory failure and/or cor pulmonale. This disease is seen exclusively in East Asians and the 5 year survival rate is <50%.9 Administration of a 14-member ring macrolide for several months was found to induce complete remission in most DPB patients.10 The fact that P. aeruginosa is not susceptible to these macrolides at clinically achievable concentrations suggests that their anti-inflammatory activity contributed primarily to the successful treatment of DPB.11 Subsequent studies have shown that macrolides suppress the production of several pro-inflammatory cytokines by bronchial epithelial cells,3,7,8 neutrophils,5 lymphocytes and monocytes.1

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Interleukin-8 (IL-8) is a member of the CXC chemokines and is mainly produced by activated monocytes. In Gram-negative infections, lipopolysaccharide (LPS) activates monocytes,12,13 Monocytes, in turn, produce IL-8 and thus attract inflammatory cells to the site of infection.13–17 It has been proposed that IL-8 is the chemokine responsible for the maintenance of chronic inflammation in DPB,3,5,8,18,19 in gastritis reported that IL-8 is the chemokine responsible for the main-}

{negative infections, lipopolysaccharide (LPS) activates mono-

{cytes.12,13 Monocytes, in turn, produce IL-8 and thus attract

{epithelial cells stimulated by pro-inflammatory cytokines,5–7

{Recent studies have shown that macrolides suppress IL-8 production by bronchial epithelial cells,30,31 Recently, LPS has been shown to stimulate the Toll-like receptor 4, which eventually activates AP-1 and NF-κB.32 Therefore, AP-1 and NF-κB play major roles in the cellular reaction in inflammation. The effects of macrolides on AP-1 and NF-κB in LPS-stimulated monocytes need to be explored.

Here we report that clarithromycin decreases IL-8 production by LPS-stimulated monocytes and suppresses IL-8 mRNA expression through NF-κB and AP-1.

Materials and methods

Reagents

LPS, benzamidine, aprotinin, leupeptin, phenylmethylsulphonyl fluoride (PMSF) and RPMI 1640 medium were purchased from Sigma Chemical Co. (St Louis, MO, USA). RPMI 1640 medium and fetal calf serum (FCS; Gibco-BRL, Life Technologies, Grand Island, NY, USA) were confirmed to contain <0.01 EU/mL endotoxin by the Limulus amoebocyte lysate test (Bio Whittaker Inc., Walkersville, MD, USA). Clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan) was dissolved in ethanol to make a stock solution (2 mg/mL).

Human peripheral blood monocytes and a human acute monocytic leukaemia cell line, THP-1

Peripheral blood was drawn from healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on a Ficoll–sodium diatrizoate solution (Ficoll-Paque; Pharmacia Biotech, Uppsala, Sweden) and were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS. PBMCs were then plated on FCS-coated plates and incubated for 1 h at 37°C in a humidified 95% air/5% CO2 atmosphere. Non-adherent cells were removed by washing with PBS. More than 90% of the adherent cells were morphologically identified as monocytes. A human acute monocytic leukaemia cell line, THP-1, was obtained from the Cell Resource Centre for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, and was maintained in RPMI 1640 medium with 10% heat-inactivated FCS without antibiotics at 37°C under a 95% air/5% CO2 atmosphere.

Bacterial strains and isolation of bacterial lysates

Escherichia coli was isolated from a clinical sample in this laboratory. P. aeruginosa PA01 strain was obtained from the American Type Culture Collection (no. 39018). A bacterial lysate of H. pylori was purchased from Biodesign International (Saco, ME, USA). E. coli was cultured overnight in Luria–Bertani broth at 37°C and P. aeruginosa was cultured in Mueller–Hinton broth. Cells were collected by centrifugation at 2000g for 10 min. The cells were lysed in 300 µL of B-PER Reagent (Pierce, Rockford, IL, USA), centrifuged at 6000g for 5 min, and the supernatant (lysate) was kept at −80°C.

Plasmids

A series of plasmids containing a serially deleted IL-8 promoter fragment [pIL-8(–1481) luc, pIL-8(–391) Luc, pIL-8(–335) Luc, pIL-8(–130) Luc, pIL-8(–112) Luc and promoterless luciferase plasmid pIL-8(0)]33 was generously provided by Dr H. Nakamura (Yamagata University School of Medicine). An NF-κB reporter plasmid, ELAM-Luc,34 was a gift from Dr M. Fenton (Boston University School of Medicine, Boston, MA, USA). An enhancerless promoter fragment of the cytomegalovirus (CMV) immediate early gene (GenBank accession no. X03922: nt 1066–1148) was isolated from pRES (Clontech, Palo Alto, CA, USA) and placed upstream of the luciferase gene in pGL3-Basic (Promega, Madison, WI, USA) to make pMiniCMV-luc. Either the AP-1 binding site (−130 to −107) or the NF-κB binding site (−106 to −65), or both binding sites from the IL-8 promoter, were placed upstream of the mini-CMV promoter in pMiniCMV-luc to make p(AP-1)-(NF-κB)-MiniCMV-luc. An AP-1 reporter plasmid containing multiple copies of a typical AP-1 binding sequence (pAP-1-Luc), an NF-κB reporter plasmid containing multiple copies of a typical NF-κB binding sequence (pNF-κB-Luc) and a negative control vector without any binding sequences (pLuc-MCS) were purchased from Stratagene (La Jolla, CA, USA).
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Measurement of IL-8 levels in culture supernatants

Human peripheral monocytes or THP-1 cells were incubated in a culture medium containing a bacterial lysate (final concentration of bacterial protein = 1 mg/L) or LPS (1 mg/L) with or without clarithromycin (10 mg/L). After 6 h, the IL-8 protein concentration in the culture medium was measured by an IL-8-specific, sandwich enzyme-linked immunosorbent assay (ELISA; BioSource International Inc., Camarillo, CA, USA).

DNA transfection and luciferase assay

Individual plasmids, together with a Renilla luciferase expression plasmid [pRL-TK: an internal control to indicate the total cellular transcription level (Promega)], were co-transfected into THP-1 cells using the Effectene Transfection Reagent (Qiagen GmbH, Hilden, Germany). After 48 h, the cells were plated into a 96-well plate (8 × 10⁵ cells per well) and exposed to LPS (0 or 1 mg/L) and clarithromycin (0 or 10 mg/L) in RPMI 1640 medium with 10% FCS. After 4 h, cells were harvested and the luciferase (i.e. firefly luciferase) activity and the Renilla luciferase activity were measured using the Dual-Luciferase reporter assay system (Promega). The luciferase activity was normalized by the Renilla luciferase activity to calculate the relative luciferase activity.

Extraction of nuclear proteins

THP-1 cells (2 × 10⁵) were incubated with LPS (0 or 1 mg/L) and clarithromycin (0 or 1 mg/L) for 4 h. Cells were washed twice with PBS and were collected by centrifugation at 500g at 4°C for 5 min. After lysing the cell membranes by the cytoplasmic extraction reagent (Pierce) containing 0.5 mg/mL benzamidine, 2 mg/L aprotinin, 2 mg/L leupeptin and 0.75 mM PMSF, the cell nuclei were collected by centrifugation at 5000g at 4°C for 5 min. Nuclear membranes were lysed in nuclear extraction reagent (Pierce) containing 0.5 mg/mL benzamidine, 2 mg/L aprotinin, 2 mg/L leupeptin and 2 mM PMSF, and centrifuged at 5000g at 4°C for 10 min. The supernatant (nuclear extract) was aliquoted and kept at −80°C. Protein concentration was determined using the BCA protein assay reagent (Pierce).

Electromobility shift assay (EMSA)

EMSA was carried out using the Gel shift assay system (Promega). The AP-1 probe (5′-AGTGTGATGACTCAGTTTGGTGGTTCTGACA-3′) and the NF-κB probe (5′-GGATCCAGGGCCTTTTCCCCAGTTAAGCG-3′) have the putative AP-1 or NF-κB binding sequences from the IL-8 promoter (underlined). Since the putative NF-κB binding sequence is not a typical binding sequence, we tested the consensus NF-κB probe (5′-GGATCCAGGGACCTTTTCCCCAGTTAAGCG-3′) as well. The probes were end-labelled with [γ-32P]adenosine triphosphate using T4 polynucleotide kinase and were purified by a G-25 spin column (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Nuclear extracts containing 6 μg of protein were incubated in a reaction mixture containing 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM MgCl₂, 0.5 mM DTT, 4% glycerol and 0.05 mg/mL poly(deoxyinosinosine-deoxyctosine) for 10 min, and subsequently incubated with the same buffer containing 35 fmol of the labelled probe at room temperature for 20 min. In the competition experiments, 1.75 pmol of the unlabelled probe or of the unlabelled mutant probe were added together with the labelled oligo. Mutant probes used were the mutant AP-1 (5′-AGTGTGATGACTCGTTTGGTGGTTCTGACA-3′), the mutant NF-κB (5′-GGATCCAGGGGACTTTCCCCTAGG-3′; IL-8 promoter specific) and mutant consensus NF-κB (5′-GGATCCAGGGGACTTTCCCCCTAGG-3′; consensus). In the inhibition experiments, nuclear extracts were preincubated with 1 μL of antibodies against human c-Fos, c-Jun, Jun B, Jun D (for AP-1), NF-κB p50 or NF-κB p65 (for NF-κB) (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) before adding the radiolabelled AP-1 or NF-κB probe. Samples were electrophoresed in a 6% DNA retardation gel (Invitrogen Co., Carlsbad, CA, USA) in 0.5× Tris borate–EDTA (TBE) buffer at 300 V for 30 min. The gel was dried and subjected to autoradiography.

Statistical analysis

Data are expressed as means ± S.D. The significant difference between the means was tested by Student’s two-tailed t test. A P value of <0.05 was considered statistically significant.

Results

Bacterial lysates from E. coli, P. aeruginosa PA01 and H. pylori all increased IL-8 production by human monocytes and THP-1 cells. In all cases, clarithromycin suppressed IL-8 production in a dose-dependent manner (51.5–66.4% inhibition at 10 mg/L) (Figure 1a).

As LPS is the primary cellular component that induces IL-8 production by human monocytes,12–14 we tested E. coli-derived LPS instead of bacterial lysates. LPS induced IL-8 production, which was suppressed by clarithromycin in both monocytes and THP-1 cells (49.3–75.0% inhibition at 10 mg/L) (Figure 1b). These data indicate that clarithromycin suppresses the signalling that transmits the LPS stimulus into the cell,32 and thus suppresses IL-8 production by monocytes and THP-1. To further explore the mechanism of action of LPS and clarithromycin, we used E. coli-derived LPS and THP-1 cells.

Clarithromycin suppresses the IL-1- or tumour necrosis factor-α (TNF-α) induced IL-8 mRNA expression in human bronchial epithelial cells,7,30 indicating that the IL-8 promoter is one of the main targets of clarithromycin. We investigated the effect of LPS and clarithromycin on the IL-8 promoter
using IL-8 promoter-luciferase constructs transfected into THP-1 cells. LPS increased the IL-8 promoter activity, and clarithromycin antagonized LPS (see pNAF in Figure 2). This is consistent with the result shown in Figure 1, and shows that LPS and clarithromycin affected the production of IL-8 at the mRNA transcription level. Experiments using serial deletion mutants showed the following: (i) the presence of the NF-κB binding sequence was necessary for LPS and clarithromycin responsiveness of the promoter (see pN112 in Figure 2); (ii) the presence of the AP-1 binding sequence enhanced both LPS and clarithromycin responsiveness (pN130 in Figure 2); and (iii) the sequence upstream of –130 altered the magnitude of the responses without changing the proportions among the responses (pN335 and pN391 in Figure 2), indicating the presence of the enhancer sequences. We conclude from these data that the AP-1 and NF-κB binding sequences are the key elements responsible for the LPS and clarithromycin responsiveness of the IL-8 promoter.

To confirm the role of the AP-1 and NF-κB binding sequences, we studied whether these sequences confer LPS and clarithromycin responsiveness to an irrelevant promoter. Either one or both of these sequences were joined with the enhancerless CMV-promoter fragment (Mini-CMV promoter), and the LPS and clarithromycin responsiveness of each plasmid was investigated (Figure 3). The Mini-CMV promoter (pMiniCMV-luc) did not respond to either LPS or
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Figure 2. Effects of clarithromycin (CAM) and LPS on serially deleted IL-8 promoter fragments in THP-1 cells. (a) A schematic presentation of the structure of the IL-8 promoter. Positions of putative binding sequences for the transcription factors are shown. HNF-1, hepatocyte nuclear factor-1; GRE, glucocorticoid responsible element; AP-1, AP-1 binding sequence; NF-κB, NF-κB binding sequence; exon 1, exon 1 of the IL-8 gene. Positions of transcription initiation (+1) and translation initiation (+44) are marked. (b) Luciferase assay. Individual deletion fragments of the IL-8 promoter were placed upstream of the luciferase gene. Each construct was co-transfected with pRL-TK (a Renilla luciferase plasmid for an internal control) into THP-1 cells. After 48 h, the cells were treated with LPS (0 or 1 mg/L) and CAM (0 or 10 mg/L) for 4 h, and both the luciferase activity and the Renilla luciferase activity were measured. The luciferase activity was normalized by the Renilla luciferase activity to calculate the relative luciferase activity. Left, the structure of the IL-8 promoter tested. Right, the relative luciferase activity for each construct under the indicated conditions. Values are means ± s.d. from four experiments. Luciferase activities significantly different from that of E. coli derived LPS (+), CAM (−) condition (black bars) are marked as *** \(P < 0.001\).

clarithromycin. Addition of either of the binding sequences conferred the LPS and clarithromycin responsiveness [p(AP-1)-MiniCMV-luc and p(NF-κB)-MiniCMV-luc]. The effects of the binding sequences were synergic, as shown by the highest response to LPS and the strongest suppression rate by clarithromycin in the experiment with p(AP-1)(NF-κB)-(Mini-CMV)-Luc. These results were consistent with the previous results (Figure 2). We conclude that both the AP-1 and NF-κB binding sequences are responsible for the LPS and clarithromycin responsiveness and that their effects are synergic.

To confirm that the factors which interact with the AP-1 or NF-κB binding sequences are actually AP-1 or NF-κB, and to examine the effects of LPS and clarithromycin on the factors, we carried out EMSAs using oligonucleotide probes that have the AP-1 (the AP-1 probe) and the NF-κB (the NF-κB probe) sequences of the IL-8 gene. The NF-κB binding sequence is not the typical NF-κB binding sequence. Therefore, we tested the consensus NF-κB sequence (the consensus NF-κB probe) as well.

Nuclear extract from the untreated THP-1 cells showed a weak binding to the AP-1 probe (Figure 4a). LPS treatment significantly increased the binding. The binding was inhibited by an excess of the unlabelled AP-1 probe, but not by the unlabelled mutant AP-1 probe. Clarithromycin suppressed the binding induced by LPS. This result shows that the binding...
was specific to the AP-1 probe, and the binding activity parallels the luciferase activity in Figure 3. Similar results were obtained in the experiments using the NF-κB probe, or the consensus NF-κB probe (Figure 4b and c).

Next we studied whether anti-AP-1 or anti-NF-κB antibodies inhibit the specific binding. The binding to the AP-1 probe was inhibited by antibodies against c-Fos, c-Jun, Jun B and Jun D, which are the component molecules of AP-1 (Figure 5a). This shows that the nuclear factor that bound to the AP-1 probe was AP-1.35 Similar results were obtained in the experiments using the NF-κB probe and antibodies against NF-κB p50 and NF-κB p65, which are the component molecules of NF-κB (Figure 5b). We conclude that the effects of LPS and clarithromycin on IL-8 expression are mediated by both AP-1 and NF-κB.

In addition to the IL-8 gene, LPS and clarithromycin may affect the expression of other genes that have AP-1 or NF-κB binding sequences in their promoters. We explored this possibility by investigating two synthetic promoters (pAP-1-Luc and pNF-κB-Luc) and a naturally occurring promoter (ELAM-luc). pAP-1-Luc and pNF-κB-Luc have multiple copies of typical AP-1 and NF-κB binding sequences, respectively. ELAM-luc has the mouse E-selectin promoter, which has a NF-κB binding sequence. For all promoters, LPS increased the luciferase activity and clarithromycin antagonized LPS (Figure 6). This result indicates that LPS and clarithromycin may affect not only the expression of the IL-8 gene, but also the expression of other genes that are regulated by AP-1 or NF-κB.

**Discussion**

Long-term macrolide therapy was so effective in DPB that it was promptly established as a standard therapy for the disease.10 It was then proved effective for chronic infections in the head and neck region such as nasal polyposis or chronic sinusitis.23 For the treatment of these diseases the anti-inflammatory effect of macrolides is the primary contributor.

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**Figure 3.** LPS and clarithromycin (CAM) responsiveness to an irrelevant promoter. The AP-1 binding sequence (–130 to –107) and/or the NF-κB binding sequence (–106 to –65) from the IL-8 promoter were placed upstream of the enhancerless CMV promoter (mini-CMV) to make constructs. The effects of LPS (0 or 1 mg/L) and CAM (0 or 10 mg/L) on the individual constructs were studied. Experiments were carried out as in Figure 2. Left, the structures of the promoter constructs studied. Right, the relative luciferase activity for each construct under the indicated conditions. Values are means ± S.D. from four experiments. Luciferase activities significantly different from that of *E. coli* derived LPS (+), CAM (−) condition (black bars) are marked as *** *P* < 0.001.
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(a) AP-1 (IL-8-specific probe)

(b) NF-κB (IL-8-specific oligo)

Figure 4. EMSAs showing the specific binding of the nuclear factors to the AP-1 probe, to the NF-κB probe or to the consensus NF-κB probe.

(a) Binding to the AP-1 probe. The radiolabelled AP-1 probe was incubated with nuclear extracts from THP-1 cells treated with LPS (1 mg/L) or LPS + clarithromycin (CAM) (10 mg/L). The AP-1 probe–nuclear factor complexes were then separated on a gel. The radiolabelled AP-1 probe was incubated without nuclear extract (lane 1), with nuclear extract from the untreated cells (lane 2), with nuclear extract from cells incubated with LPS (lanes 3–5) or with nuclear extract from cells incubated with LPS and CAM (lanes 6–8). In lanes 4, 5, 7 and 8, the unradiolabelled (i.e. cold) probes were added to the reactions to test whether they compete with the radiolabelled AP-1 probe. Arrows indicate the specific bands.

(b) Binding to the NF-κB probe. Experiments were carried out as in (a) using the NF-κB probe that contains the binding sequences from the IL-8 promoter. An arrow indicates the specific band.

(c) Binding to the consensus NF-κB probe. Experiments were carried out as in (b).
Application in the treatment of skin diseases such as psoriasis vulgaris and palmoplantar pustulosis has been reported.\textsuperscript{24,25}

To investigate other diseases in which the anti-inflammatory effect of macrolides might be beneficial, it is important to identify the types of cells on which macrolides exert this effect. In Gram-negative infections, monocytes produce IL-8 in response to LPS, whereas epithelial cells do not.\textsuperscript{26} Epithelial cells secondarily produce IL-8 in response to IL-1\(\alpha\), IL-1\(\beta\) or TNF-\(\alpha\), which are the products of LPS-stimulated monocytes. Therefore, if macrolides do not act on LPS-stimulated monocytes, they are unlikely to exert the anti-inflammatory effect in such infections. Nevertheless, information on the effects of macrolides on LPS-stimulated monocytes has not been available. We observed that most of the DNA transfection methods currently available are incapable of transiently introducing DNA into monocytes or into the monocytic cell line THP-1 at a satisfactory efficiency, and such difficulty may hinder the experiments. A transfection reagent that has recently become available enabled us and others to use THP-1 cells (http://www.qiagen.com/transfectiontools/transquest/index.asp). Our results, together with those previously reported on epithelial cells,\textsuperscript{30} show that clarithromycin acts on both monocytes and epithelial cells, indicating that clarithromycin may efficiently suppress IL-8 production at the sites of infection.

Our results present a hypothesis that macrolides may be effective for more chronic inflammatory diseases than described above, especially in those where Gram-negative bacteria are involved, such as \textit{H. pylori}-associated chronic gastritis. Several studies have demonstrated that LPS from \textit{H. pylori} stimulates gastric epithelial cells to produce IL-8, which in turn elicits chronic gastritis.\textsuperscript{20,22,27} Amoxicillin, tetracycline, metronidazole and macrolides (mainly clarithromycin) in combination with proton pump inhibitors or bismuth salts comprise the standard therapy.\textsuperscript{36,37} Our results indicate that clarithromycin may ameliorate inflammation caused by LPS from \textit{H. pylori} through its anti-inflammatory activity. Although clarithromycin-resistant \textit{H. pylori} strains have been reported, clarithromycin may still be beneficial owing to its anti-inflammatory activity.

We have shown that clarithromycin suppresses the LPS signalling that leads to both AP-1 and NF-\(\kappa\)B. Others have shown that clarithromycin suppresses TNF-\(\alpha\) and IL-1\(\beta\) signalling leading to both AP-1 and NF-\(\kappa\)B.\textsuperscript{31} These results
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indicate that clarithromycin may act in the pathway(s) after the point where the pathway from LPS and the pathway from TNF-α and IL-1β converge.

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References


