Salicylates Inhibit Lipopolysaccharide-Induced Transcriptional Activation of the Tissue Factor Gene in Human Monocytic Cells

By Paul Oeth and Nigel Mackman

Binding of plasma Factor VII/Vila to the tissue factor (TF) receptor initiates the coagulation protease cascades. TF expression by circulating monocytes is associated with thrombotic and inflammatory complications in a variety of diseases. Transcriptional activation of the human TF gene in monocytic cells exposed to bacterial lipopolysaccharide (LPS) is mediated by binding of c-Rel/p65 heterodimers to a κB site in the TF promoter. Here, we report that a family of anti-inflammatory agents, known as the salicylates, inhibited LPS induction of TF activity and TF gene transcription in human monocytes and monocytic THP-1 cells at clinically relevant doses. Furthermore, sodium salicylate blocked the LPS-induced proteolytic degradation of IκBα, which prevented the nuclear translocation of c-Rel/p65 heterodimers. In contrast, two other nonsteroidal anti-inflammatory drugs, ibuprofen and indomethacin, did not inhibit LPS induction of the TF gene. These results indicated that salicylates inhibited LPS induction of TF gene transcription in monocytic cells by preventing nuclear translocation of c-Rel/p65 heterodimers. The clinical benefits of salicylates in the treatment of several diseases, including atherosclerosis and rheumatoid arthritis, may be related to their ability to reduce monocyte gene expression.

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In this study, we showed that NaSal, salsalate, and aspirin all inhibited LPS induction of TF gene expression in human monocytic cells in a dose-dependent manner. All three agents blocked LPS-induced nuclear translocation of c-Rel/p65 heterodimers, indicating that they inhibited TF expression by preventing transcriptional activation of the TF gene.

MATERIALS AND METHODS

Materials. LPS (Escherichia coli serotype O111:B4) was purchased from Calbiochem (San Diego, CA). Phorbol 12-myristate 13-acetate (PMA), acetylsalicylic acid, ibuprofen, and indomethacin were obtained from Sigma (St Louis, MO). NaSal and salsalate were purchased from Aldrich Chemical (Milwaukee, WI). A fluorescein isothiocyanate (FITC)-labeled conjugate of MY4 (MY4-FITC), a murine monoclonal antibody to human CD14, was purchased from Coulter Immunology (Hialeah, FL). An FITC-labeled antihuman TF antibody, TF8-5G97, was kindly provided by T.S. Edgington (The Scripps Research Institute, La Jolla, CA). Cell viability and toxicity. Cell viability was determined using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega Corp, Madison, WI). Briefly, 5 x 10^5 cells were treated and transferred to a 96-well plate (Costar, Cambridge, MA) in a volume of 100 μL. A total of 20 μL of the MTS/PM5 solution was added to each sample, and the plate was incubated at 37°C, in 5% CO2 for 5 hours. The plate was then read at 490 nm on an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Devices, Menlo Park, CA). Cell viability was also assessed using trypan blue (Sigma). 35S-translabeled incorporation was conducted as follows: 2 x 10^6 cells were pelleted at 300g for 15 minutes and then resuspended in RPMI 1640 medium without cysteine or methionine (Bio-Whittaker Inc, Walkersville, MD) and trans35S metabolic labeling reagent (ICN Pharmaceuticals Inc, Costa Mesa, CA) to a final concentration of 1 μCi/mL. After treatment, cells were pelleted at 300g, washed once with phosphate-buffered saline, and resuspended in 100 μL of bovine serum albumins (1 mg/mL). Trichloroacetic acid precipitable counts were counted in the presence of 2 mL of liquid scintillant (Ultima Gold) by a Beta counter (Beckman Instruments, Irvine, CA).

Monocyte isolation and cell culture. Heparinized blood was drawn from volunteers, and plasma was removed after centrifugation at 200g. Blood cells were diluted 1:1 (vol:vol) in endotoxin-free RPMI 1640 medium (Bio-Whittaker) and were overlaid onto Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). After centrifugation at 400g, the peripheral blood mononuclear cells (PBMCs) were removed and washed twice with medium and resuspended in RPMI 1640 medium (BioWhittaker, Inc) and were overlaid onto RPMI 1640 media with 2% fetal calf serum (Gemini Bioproducts, Calabasas, CA). Human mononuclear cells were obtained from the American Type Culture Collection (Rockville, MD) and were cultivated at a density of 2 to 5 x 10^6 cells/mL as described. All reagents were tested by Whittaker Inc (Walkersville, MD) and contained less than 0.01 ng/mL endotoxin contamination.

TF activity. Cell pellets were solubilized at 37°C for 15 minutes using 15 mmol/L octyl β-D-glucopyranoside. TF activity was assayed in cell lysates using a one-stage clotting assay as described. Clotting times were converted to milliunits of TF activity by comparison with a standard curve established with purified human brain TF. For reference, a clotting time of 50 seconds corresponds to 1,000 mU of TF activity.

Fluorescence activated cell sorting (FACS). PBMCs (1 x 10^6 cells/mL) were stimulated for 5 hours with 10 ng/mL LPS before incubation of cells for 30 minutes at 4°C with antihuman TF-FITC or MY4-FITC antibodies. Cells were then fixed in 0.5% paraformaldehyde and were analyzed by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). Live gating on the monocye population was accomplished using near forward angle and orthogonal light scatter as well as staining with MY4-FITC antibody.

Analysis of TF mRNA. Total cellular RNA, purified using TRI-ZOL reagent (GIBCO-BRL, Gaithersburg, MD) was analyzed by Northern blotting as described. A 641-bp human TF cDNA fragment was labeled with [α32P]deoxyctydine triphosphate (ICN) using the Prime It Kit (Stratagene, La Jolla, CA) and was used to determine TF mRNA levels. An egr-1 cDNA fragment was kindly provided by E. Adamson (La Jolla Cancer Research Foundation, La Jolla, CA). Blots were rehybridized with the housekeeping genes, glucose-6-phosphate dehydrogenase (a gift from G. Parry, The Scripps Research Institute) or glyceraldehyde 3-phosphate dehydrogenase (G3PDH; Clontech Laboratories, Inc, Palo Alto, CA). Autoradiography was performed at -70°C, using Kodak XAR-5 film with intensifying screens (Eastman-Kodak, Rochester, NY). Band intensities were quantified by densitometric analysis using a Phosphimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA) or Personal Densitometer (Molecular Dynamics).

Nuclear run-on. Nuclear run-on assays were performed using a modification of a procedure described previously. Cells were unstimulated or LPS (10 μg/mL)-stimulated (for 1 hour) with or without a 15-minute pretreatment with NaSal (16 mmol/L). Cells (5 x 10^6) were lysed in a homogenizer and nuclei were collected using a sucrose (2.0 mol/L) cushion. Nuclear RNA was labeled using [α35P] uridine triphosphate (UTP), and samples were treated sequentially with DNase 1 (final concentration, 1 U/μL) for 10 minutes at 37°C and with proteinase K (final concentration, 200 μg/mL) for 45
minutes at 37°C. Samples were extracted with phenol/chloroform and were ethanol-precipitated, and nuclear RNA was purified using a G-50 column. Prehybridization, hybridization, and washing of the filters were performed as described. Radioactivity was quantified using a Phosphorimager and ImageQuant software (Molecular Dynamics).

**Plasmids and transfections.** TF, G3PDH and tumor necrosis factor-α (TNFα) cDNAs present in pGEM3Z (Promega Corp), pSP73 (Promega Corp), and pSP64, respectively, were used in the nuclear run-on assays. pTF(-278)LUC contains 278 bp of the human TF promoter cloned upstream of the luciferase reporter gene and p(TF)SVLUC contains four copies of the TF eB site cloned upstream of the simian virus 40 promoter encoding the luciferase reporter gene. Cells were transfected using diethyl aminoethyl-dextran (underlined). 5'-GTCGCCAGTTTTCCTACCGGG-3', was annealed with a complimentary primer and was radiolabeled using [α-32P]deoxytidephosphate (ICN) as described. Similarly, binding of the transcription factor, EGR-1, was performed using a radiolabeled oligonucleotide (Operon Tech) containing a prototypic EGR-1 site (underlined), 5'-CGCGCCGCGGGCGATTTGAGTCA-3'. Protein-DNA complexes were separated from free DNA probe by electrophoresis through 6% nondenaturing acrylamide gels (Novex, San Diego, CA) in 0.5× Tris borate-EDTA. Band intensities were quantified by densitometric analysis using a Personal Densitometer and ImageQuant software (Molecular Dynamics).

**RESULTS**

Salicylates inhibit LPS-induced TF expression in human mononuclear cells. NaSal and aspirin are able to inhibit NF-κB activation, as was recently observed. We have shown that LPS induction of the TF gene requires activation of c-Rel/p65 heterodimers in human mononuclear cells. Therefore, we examined the ability of various salicylates and other NSAIDs to inhibit LPS-induced TF expression in both PBMCs and mononuclear THP-1 cells. Cells were pretreated for 15 minutes with NaSal (10 mmol/L), salsalate (10 mmol/L), aspirin (10 mmol/L), ibuprofen (200 μmol/L), or indomethacin (25 μmol/L) before LPS stimulation for 5 hours. NaSal, salsalate, and aspirin all inhibited LPS induction of TF activity (mean ± SD) is shown from three independent experiments.

![Graph showing inhibition of LPS-induced TF activity by NaSal](https://example.com/graph.png)

**Fig 2.** NaSal inhibition of LPS-induced TF activity in human mononuclear cells. (A) PBMCs were exposed to LPS (10 ng/mL) for 5 hours with or without a 15-minute pretreatment with NaSal at doses ranging from 0.1 to 10 mmol/L. (B) THP-1 cells were exposed to LPS (10 μg/mL) for 5 hours with or without a 15-minute pretreatment with NaSal at doses ranging from 0.1 to 10 mmol/L. Control samples were incubated for 5 hours without LPS or NaSal. TF activity (mean ± SD) is shown from three independent experiments.

Addition of NaSal

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![Graph showing effect of NaSal addition before and after LPS stimulation](https://example.com/graph.png)

**Fig 3.** Effect of addition of NaSal before and after LPS stimulation. THP-1 cells were incubated with NaSal (10 μmol/L) before and after LPS stimulation for 5 hours. The NaSal inhibition (%) of LPS-induced TF activity is shown for a typical experiment. Similar results were observed in two independent experiments.
SALICYLATES INHIBIT TISSUE FACTOR EXPRESSION

Fig 4. NaSal inhibition of LPS-induced TF antigen expression in human monocytes. PBMCs were incubated with LPS (10 ng/mL) for 5 hours with or without a 15-minute pretreatment with NaSal (0.1 and 10 mmol/L). The control sample is PBMCs incubated for 5 hours without LPS or NaSal. Cells were stained with an FITC-labeled human TF-specific monoclonal antibody, TF8-509. Expression of TF on the cell surface was determined by fluorescence-activated cell sorting (FACS) using live gating to analyze the monocyte population. Results from a representative experiment are expressed as log fluorescence intensity versus cell number. Mean channel fluorescence (MCF) for each sample is shown.

TF activity in PBMCs and monocyteic THP-1 cells, whereas ibuprofen and indomethacin had no significant effect (Fig 1). These results indicated that both nonacetylated and acetylated salicylates inhibited LPS-induced TF expression.

We next examined the effect of various doses of NaSal on LPS-induced TF activity. Both PBMCs and THP-1 cells were pretreated for 15 minutes with NaSal (0.1 to 10 mmol/L) before LPS stimulation for 5 hours to maximally induce TF activity. NaSal inhibited LPS induction of TF activity in PBMCs and monocyteic THP-1 cells in a dose-dependent manner (Figs 2A and B). Similar results were observed using aspirin and salsalate (data not shown). Time course experiments analyzed the effect of NaSal (10 mmol/L) at different times (2, 5, 8, and 24 hours) after LPS stimulation. These results showed that NaSal abolished LPS induction of TF activity at all time points examined (data not shown), indicating that NaSal did not act by simply delaying the induction of TF expression.

The level of inhibition after the addition of NaSal at various times before and after LPS stimulation was also examined. LPS induction of TF activity was abolished by preincubating cells with NaSal (10 mmol/L) for between 0 and 120 minutes before LPS stimulation (Fig 3). In addition, longer preincubation times (60 to 120 minutes) with lower concentrations of NaSal (0.5 to 1.0 mmol/L) did not increase the level of inhibition (data not shown). However, there was a time-dependent loss of inhibitory activity when NaSal was added after LPS stimulation (Fig 3). The inhibitory activity required the continual presence of NaSal because full LPS-induced TF activity was recovered when NaSal was removed before LPS stimulation (data not shown). The effect of NaSal was not a result of inhibition of procoagulant activity in the one-stage clotting assay, because this inhibitor had no effect on clotting times when added to cells immediately before harvest (data not shown).

LPS induction of cell-surface TF expression in PBMCs was measured to determine if NaSal inhibited increases in TF protein expression (Fig 4). PBMCs were pretreated with NaSal (0.1 and 10 mmol/L) for 15 minutes before a 5-hour incubation with or without LPS (10 ng/mL). LPS induction of TF expression was again inhibited in a dose-dependent manner, with complete inhibition at 10 mmol/L (Fig 4).

To exclude the possibility that inhibition by NaSal was due to toxicity, we examined protein incorporation of 35S-methionine and 35S-cysteine in the presence of LPS and various concentrations of NaSal. Incorporation of these radiolabeled amino acids into newly synthesized cellular protein is a general measure of protein synthesis within a population of cells. PBMCs and THP-1 cells pretreated with NaSal and then stimulated with LPS showed no significant decrease in protein synthesis at any concentration of NaSal used in these experiments (data not shown). In addition, measurement of cell viability was assessed by MTS conversion to formazan via dehydrogenase enzymes found in metabolically active cells using a calorimetric assay. No reduction in cell viability was observed using LPS and NaSal (data not shown). Trypan blue cell counts also showed no significant reduction in cell viability (data not shown). Taken together, we observed no significant cell toxicity at the concentrations of NaSal and LPS used during the investigation.

NaSal inhibits LPS-induced TF mRNA expression. We next examined if NaSal inhibited LPS induction of TF mRNA. Before LPS stimulation for 2 hours to maximally induce TF mRNA, monocyteic THP-1 cells were pretreated for 15 minutes with NaSal at doses ranging from 0.1 to 10 mmol/L. LPS induction of TF mRNA was inhibited in a
**Fig 6.** Nuclear run-on analysis of TF gene transcription in THP-1 cells. Nuclei were isolated from unstimulated cells (control), cells stimulated with LPS (10 µg/mL) for 1 hour, and cells pretreated with NaSal (10 mM) for 15 minutes before LPS stimulation for 1 hour. Labeled nuclear RNA levels were determined by hybridization to various target cDNAs (G3PDH, TF, and TNFα) and vector controls pSP73, pGEM3Z, pSP64 (data not shown). The autoradiogram was exposed for 14 days at -80°C with intensifier screens. The numbers shown below represent band intensities quantified using a Personal Densitometer and ImageQuant software. No hybridization was detected with the other slots (-). Similar results were observed in two independent experiments.

**Fig 5.** Northern blot analysis of TF mRNA expression in THP-1 cells. (A) Total RNA was extracted from THP-1 cells exposed to LPS (10 µg/mL) for 2 hours with or without a 15-minute pretreatment with NaSal at doses ranging from 0.1 to 10 mM. The control sample was incubated without LPS or NaSal. TF mRNA levels were determined by Northern blot analysis using a radiolabeled human TF cDNA probe. The blot was reprobed to determine G3PDH mRNA levels as a measure of RNA loading. The position of the TF mRNA is indicated. Band intensities were quantified by densitometric analysis using a Personal Densitometer and ImageQuant software. Normalized levels of TF mRNA are shown. (B) THP-1 cells were exposed to PMA (50 ng/mL) for 2 hours with or without a 15-minute pretreatment with NaSal (10 mM). Control cells were incubated without PMA or NaSal. EGR-1 mRNA levels were determined by Northern blot analysis using a radiolabeled human egr-1 cDNA probe. The blot was reprobed to determine glucose 6-phosphatase dehydrogenase mRNA levels as a measure of loading. The position of the EGR-1 mRNA is indicated. No significant differences were observed between EGR-1 mRNA levels in RNA prepared from PMA-treated cells or NaSal- and PMA-treated cells. Similar results were obtained in an independent experiment.

dose-dependent manner by NaSal (Fig 5A). To exclude the possibility that NaSal was a general transcriptional inhibitor of THP-1 cells, we analyzed PMA induction of another gene, egr-1, which is regulated by a κB-independent mechanism. The egr-1 gene expresses a nuclear phosphoprotein that binds to DNA in a zinc-dependent manner. PMA induction of EGR-1 mRNA expression was not affected by NaSal (10 mM; see Fig 5B), indicating that NaSal was not acting as a general inhibitor of transcriptional activation in these monocytic cells.

**NaSal inhibits LPS induction of TF gene transcription and the human TF promoter.** To determine if NaSal blocked LPS induction of TF gene transcription, nuclear run-on experiments were performed. LPS stimulation of THP-1 cells transiently increased the rate of TF gene transcription at 1 hour (Fig 6). This increase was abolished by pretreating the cells with NaSal (Fig 6). Similarly, NaSal abolished the LPS-stimulated increase in TNF-α gene transcription (Fig 6), which is also regulated by NF-κB/Rel proteins. In contrast, the rate of transcription of the housekeeping gene, G3PDH, was not altered by LPS stimulation or NaSal and LPS (Fig 6).
To further analyze the NaSal inhibition of TF gene transcription, THP-1 cells were transiently transfected with a plasmid, pTF(-278)LUC, which contains 278 bp of the human TF promoter cloned upstream of a luciferase reporter gene, and a plasmid, (p(TF))_4 SVLUC, which contains four copies of the TF κB site cloned upstream of the minimal simian virus 40 promoter expressing the luciferase gene. Previous studies have shown that LPS induction of the TF promoter is mediated by an LPS response element (−227 to −172 bp) in the wild-type TF promoter or by tandem copies of the isolated TF κB site. Before a 5-hour LPS stimulation to maximally induce luciferase expression, transfected cells were pretreated for 15 minutes with NaSal in doses ranging from 0.1 to 10 mmol/L. LPS induction of luciferase activity expressed by pTF(-278)LUC and p(TF))_4 SVLUC was inhibited in a dose-dependent manner by NaSal (Fig 7).

Salicylates inhibit LPS activation of c-Rel/p65 heterodimers. To examine if NaSal acted by preventing activation of c-Rel/p65 heterodimers, THP-1 cells were pretreated for 15 minutes with NaSal (0.1 to 10 mmol/L) before a 2-hour LPS stimulation. Nuclear extracts were analyzed by EMSA using a radiolabeled oligonucleotide containing the TF κB site. LPS activation of c-Rel/p65 heterodimers in THP-1 cells was inhibited by NaSal in a dose-dependent manner (Fig 8A). NaSal also inhibited the LPS activation of NF-κB(p50/p65) in THP-1 cells (data not shown). In addition, Western blots showed that NaSal prevented the reduction in...
IkBα protein in the cytosol 1 hour after LPS stimulation (Fig 8B). Similar results were observed in cells stimulated with LPS for 2 hours (data not shown). These results indicated that NaSal inhibited LPS induction of TF gene expression in human monocytic cells by preventing proteolytic degradation of IkBα protein and nuclear translocation of c-Rel/p65 heterodimers.

To exclude the possibility that NaSal nonspecifically inhibited protein-DNA interactions, we analyzed the binding of EGR-1 to a prototypic EGR-1 site using nuclear extracts from THP-1 cells with or without NaSal. THP-1 cells were pretreated for 15 minutes with NaSal (10 mmol/L) before a 2-hour PMA (50 ng/mL) stimulation. EMSA showed that EGR-1 binding activity induced by PMA was not affected by the presence of NaSal (Fig 8C).

Other NSAIDs, including salsalate, aspirin, ibuprofen, and indomethacin, were examined to determine if these agents also inhibited the activation of c-Rel/p65 heterodimers. THP-1 cells were pretreated for 15 minutes with aspirin (10 mmol/L), NaSal (10 mmol/L), salsalate (10 mmol/L), ibuprofen (200 μmol/L), or indomethacin (25 μmol/L) and then were stimulated with LPS (10 μg/mL) for 2 hours. Aspirin, NaSal, and salsalate all abolished the nuclear translocation of c-Rel/p65 heterodimers, whereas ibuprofen and indomethacin had no effect (Fig 9), which is consistent with the inability of ibuprofen and indomethacin to inhibit LPS induction of TF activity in monocytic cells (Fig. 1).

NaSal concentrations inhibiting 50% of the LPS-induced TF expression in the different assays (ICso) were 0.656 to 1.030 mmol/L (Table 1). Differences in the ICso may be caused by the different incubation times of these assays or may reflect variations in the sensitivity of each assay to inhibition by NaSal.

**DISCUSSION**

This study showed that salicylates inhibited LPS-induced TF expression in human monocytic cells. This inhibition was observed with NaSal, salsalate, and aspirin, indicating that both nonacetylated and acetylated salicylates share a common pathway in their ability to inhibit TF expression. NaSal inhibited LPS induction of TF activity, TF mRNA, and TF gene transcription but did not prevent PMA induction of EGR-1 mRNA expression, indicating that it was not a general inhibitor of transcriptional activation. In addition, NaSal inhibited LPS-induced nuclear translocation of c-Rel/p65 heterodimers. Other NSAIDs, such as ibuprofen or indomethacin, failed to inhibit LPS-induced TF activity or activation of c-Rel/p65 heterodimers. Thus, salicylates suppressed LPS induction of the TF gene in monocytic cells by preventing activation of c-Rel/p65 heterodimers.

LPS stimulation of THP-1 cells has been shown to lead to phosphorylation of IkBα, with a corresponding activation of transcription factors containing c-Rel and p65.41 We have shown that LPS stimulation of THP-1 cells transiently reduces levels of cytosolic IkBα protein45 and that overexpression of IkBα in THP-1 cells prevents LPS induction of TF gene transcription.21 Here, we show that NaSal blocks activation of c-Rel/p65 heterodimers in monocytic cells by preventing the LPS-induced proteolytic degradation of IkBα.

TF is expressed by monocytes/macrophages in rheumatoid arthritis in which increased levels of TF are found in the synovium of patients.5 In addition, TF protein and mRNA are expressed by macrophage-derived foam cells in atherosclerotic plaques.7 Both of these diseases are treated clinically with salicylates; therefore, the ability of salicylates to inhibit TF expression by monocytes may contribute to their efficacy and antithrombotic properties.

Cell surface expression of TF by monocyte/macrophages and secretion of various cytokines, including TNFα, are associated with the pathology of Gram-negative sepsis.11,12,14 An effective clinical inhibitor of transcriptional activation of TF and cytokine genes could be useful in the treatment of sepsis. In this study, we found that NaSal inhibited LPS-induced transcription of both the TF and TNFα genes in THP-1 monocytic cells. Aspirin has previously been examined in rat and nonhuman primate models of endotoxemia with beneficial results46-48; however, no follow-up studies assessed its clinical use in sepsis therapy. Another NSAID, ibuprofen, has been extensively studied in relation to sepsis therapy.49 Ibuprofen clearly reduces certain symptoms observed during septic shock, such as increased cardiac output,
elevated mean arterial blood pressure, and end products of the cyclooxygenase pathway. In contrast, endotoxin-induced elevation of the NF-κB-regulated genes interleukin-6 and TNFα were not decreased by ibuprofen administration. Indomethacin, another NSAID, which (like ibuprofen) inhibits cyclooxygenase, was unable to inhibit transcription of the human immunodeficiency virus. Our data and those of others show that ibuprofen and indomethacin failed to inhibit activation of NF-κB/Rel proteins, which is consistent with their inability to reduce transcription of NF-κB/Rel-regulated genes. Sepsis is a complex pathological condition that will most likely require a well-defined multiple-drug treatment for effective therapy. Given the new findings in relation to the modes of action observed with salicylates, it seems their use in sepsis therapy should be reexamined. NF-κB/Rel proteins regulate the activation of many genes that are induced during inflammatory responses, including genes encoding various cytokines and adhesion molecules. Thus, inhibiting the activation of NF-κB/Rel proteins, especially in monocytes, may account for many of the anti-inflammatory effects of the salicylates.

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REFERENCES


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