Anti-Inflammatory Activity in Vitro and in Vivo of the Protein Farnesyltransferase Inhibitor Tipifarnib

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ABSTRACT
Protein farnesyltransferase inhibitors (FTIs) have shown clinical responses in hematologic malignancies, but the mechanisms are unclear. To better understand potential mechanisms of action, we have studied effects of the FTI tipifarnib on inflammatory responses in vitro and in vivo. In a human leukemia cell line THP-1, tipifarnib inhibited lipopolysaccharide (LPS)-induced transcription of chemokines [monocyte chemotactic protein (MCP)-1 and MCP-2], cytokines [interleukin (IL)-1β, IL-6, and interferon (IFN)α], signaling molecules (MyD88 and STAT-1), proteases [matrix metalloproteinase (MMP-9)], and receptors (urokinase receptor). Tipifarnib also inhibited LPS-induced secretion of MMP-9, IL-6, MCP-1, and IL-1β in THP-1 cells. In primary human peripheral blood mononuclear cells, dose-dependent inhibition of LPS-induced tumor necrosis factor (TNF)-α, IL-6, MCP-1, and IL-1β by tipifarnib was observed with no evidence of cytotoxicity. Similar results were obtained in vivo in a murine model of LPS-induced inflammation, where pretreatment with tipifarnib resulted in significant inhibition of TNF-α, IL-6, MCP-1, IL-1β, and MIP-1α production. Tipifarnib had no effect in vitro or in vivo on LPS-induced IL-8. Studies in THP-1 cells to address potential mechanism(s) showed that tipifarnib partially inhibited LPS-induced p38 phosphorylation. Tipifarnib significantly inhibited inhibitory subunit of nuclear factor-κB (NF-κB) (IκB-α) degradation and p65 nuclear translocation induced by LPS, but not by tumor necrosis factor-α, IL-1α, or toll-like receptor (TLR)2 ligand, suggesting that the target for inhibition of NF-κB activation was exclusive to the LPS/TLR4 signal pathway. The extent of IκB-α degradation inhibition did not correlate with inhibition of Ras farnesylation, indicating that Ras was not the target for the observed anti-inflammatory activity of tipifarnib. Our findings differ from those for other FTIs, which may have relevance for their dissimilar activity in specific tumor repertoires.

The links between cancer and inflammation are well established (for review, see Balkwill et al., 2005). Many human and murine cancers are found in a microenvironment rich in cytokines, chemokines, and inflammatory enzymes. It is therefore of interest to investigate the effects of antitumor drugs on inflammation. Tipifarnib [R115777, Zarnestra, (R)-6-amino[(4-chlorophenyl)(1-methyl-1H-imidazol-5-yl)methyl]-4-(3-chlorophenyl)-1-methyl(1H)-quinolinone] is an orally active inhibitor of protein farnesyltransferase. It has shown significant antitumor activity and is currently in clinical trials for acute myeloid leukemia and myelodysplastic syndrome (Cortes, 2003). Farnesyltransferase is an enzyme that catalyzes the attachment of a farnesyl group, from farnesyl pyrophosphate, to the cysteine-thiol group of protein C-terminal CAAX consensus sequences (Moores et al., 1991; Reiss et al., 1991). A variety of cellular proteins are farnesylated (Schafer and Rine, 1992; Tamanoi et al., 2001), including Ras superfamily G proteins, nuclear lamins A and B, rhodopsin kinase, centromere-binding proteins CENP-E and CENP-F, cochaperone DnaJ/HDJ-2, progestrone receptor (O’Meara and Kinsella, 2003), and cytosolic phospholipase A2 (Jenkins et al., 2003). Ras farnesylation is critical for oncogenic Ras signaling (Kato et al., 1992), and Ras mutants are associated with ~30% of human cancers. Farnesyltransferase inhibitors (FTIs) were developed to inhibit activity of protein farnesyltransferase, leading to reduced activation of oncogenic Ras proteins by preventing farnesylation. FTIs have been shown to inhibit tumor growth in vitro and in vivo, with significant clinical activity observed in hematologic malignancies. However, the mechanisms of action of FTIs are not fully understood.

Inflammation plays a significant role in cancer progression, and the immune system plays a critical role in the response to cancer therapy. Inflammation can be a double-edged sword in cancer therapy, with both anti-inflammatory and pro-inflammatory signaling pathways playing a critical role in the efficacy of cancer treatments. Anti-inflammatory agents, such as tipifarnib, have shown potential to modify the immune response and improve therapeutic outcomes.

Prominent ABBREVIATIONS: FTI, farnesyltransferase inhibitor; TNF, tumor necrosis factor; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; IL, interleukin; IL-1α, interleukin-1α receptor; TLR, toll-like receptor; TNFR, tumor necrosis factor receptor; MCP, monocyte chemotactic protein; MMP, matrix metalloproteinase; STAT, signal transducer and activator of transcription; MIP, macrophage inflammatory protein; IκB, inhibitory subunit of NF-κB; FBS, fetal bovine serum; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; ERK, extracellular signal-regulated kinase; SCH 66336, 4-(2-(4-(8-chloro-3,10-dibromo-6,11-dihydro-5H-benzo-[5,6]-cyclohepta[1,2-b]pyridin-11(6H)-yl]-1-piperidinyl)-2-oxo-ethyl)-1-piperidinecarboxamide; MEK, mitogen-activated protein kinase kinase; IKK, IκB kinase; TRAF6, tumor necrosis factor receptor-associated factor 6; Pam3CSK4, N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-[R]-Cys-[S]-Ser-[S]-Lys (4) trihydrochloride; LB 42708, 4-[[1-[[1-(4-bromophenyl)methyl]-1H-imidazol-5-yl]methyl]-4-(1-naphthalenyl)-1H-pyrrrol-3-yl]carbonyl]-9(1)1-morpholine.
prevent Ras farnesylation and cell membrane association and therefore block aberrant Ras function in cancer. However, further studies have shown that the response to FTIs does not correlate with Ras status, suggesting inhibition of farnesylation of other proteins might also contribute to the antitumor properties of FTIs (Cox and Der, 1997; Sebti and Hamilton, 2000).

Recently a FTI has been shown to inhibit TNF-α-induced NF-κB activation in vitro (Takada et al., 2004). Na et al. (2004) also showed inhibition by another FTI of LPS-induced NF-κB activation in vitro, and inducible nitric-oxide synthase, cyclooxygenase-2, TNF-α, and IL-1β expression in vivo. These findings suggest that FTIs might have anti-inflammatory activity through affecting IL-1R/TLR/TNFFR signaling. We tested the effect of tipifarnib on inflammatory responses induced by LPS. The results of our study demonstrated inhibition by tipifarnib of LPS induction of a number of inflammatory mediators, in vitro and in vivo. We present the first evidence for down-regulation by an FTI of LPS-induced MCP-1, IL-6, MMP-9, STAT1, MyD88, and MIP-1α. We also found that tipifarnib significantly inhibited IκB degradation and p65 translocation induced by LPS, but not by TNF-α, TLR2 ligand, or IL-1α. In addition, inhibition of Ras farnesylation did not correlate with the extent of inhibition of IκB degradation. The results of our study suggest that tipifarnib affected proinflammatory responses through inhibiting farnesylation of one or more proteins other than Ras that are involved in a pathway exclusive to LPS/TRLR4 signaling.

Materials and Methods

Materials. General chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Human THP-1 cells were obtained from the American Type Culture Collection (Manassas, VA). RPMI 1640 cell culture medium and penicillin-streptomycin were purchased from Sigma-Aldrich, and fetal bovine serum (FBS) was from Invitrogen (Carlsbad, CA). LPS, from Escherichia coli 026:B6, was obtained from Sigma-Aldrich. IL-1α and TNF-α were purchased from R&D Systems (Minneapolis, MN). Pam3CSK4, the synthetic lipoprotein, was purchased from InvivoGen (San Diego, CA).

Cell Culture, RNA Isolation, and Cytokine Analysis. THP-1 cells were cultured in RPMI 1640 medium containing 10% FBS, 100 units/ml penicillin, and 10 μg/ml streptomycin at 37°C under 5% CO₂. Medium components were certified to contain less than 0.3 endotoxin unit/ml. For RNA isolation, THP-1 cells were cultured at 4.5 × 10⁶/ml in 0.5% FBS/RPMI 1640 medium in the absence or presence of 100 ng/ml LPS plus or minus 5 μM tipifarnib. Cells were collected at 3, 6, and 12 h for total RNA isolation using the RNeasy mini kit from QIAGEN (Valencia, CA). For cytokine secretion studies, THP-1 cells at 3.4 × 10⁵/ml were cultured in 96-well plates in 0.5% FBS/RPMI 1640 medium, in the absence or presence of 100 ng/ml LPS plus or minus tipifarnib. Supernatants were collected at different time points for analysis of cytokine production.

Microarray Experiments and Data Analysis. Five micrograms of total RNA was linearly amplified to double-stranded cDNA using T7-based amplification. The cDNA was purified using the Qiagen 100 bp PCR purification kit (Qiagen) and used to generate amplified RNA using AmpliScribe T7 high-yield transcription kit (Epicenter Technologies, Madison, WI). Ten micrograms of purified amplified RNA was reverse transcribed to cDNA using the SuperScript RTH kit (Invitrogen) and labeled by direct incorporation of Cy5-dCTP. The generated cDNA probe was used for hybridizing to cDNA microarrays. The human cDNA microarrays were custom-made as described previously (Peterson et al., 2004) and contained approximately 8000 human genes and expressed sequence tags. RNA preparation, cDNA probe synthesis, and hybridization were performed as described by Shaw et al. (2003). Data normalization and preparation were performed as described previously (Peterson et al., 2004).

Reverse Transcriptase and Real-Time Quantitative Polymerase Chain Reaction. DNase-treated total RNA (2–3 μg) was reverse transcribed to cDNA using the Transcriptor first strand cDNA synthesis kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions. Real-time PCR was carried out with the LightCycler FastStart DNA MasterPLUS SYBR Green I kit (Roche Diagnostics) according to the manufacturer’s instructions. Real-time PCR reaction conditions were as follows. The 20-μl final volume contained 4 mM MgCl₂, 0.5 μM of each primer, 2 μl of Master mix, and 2 μl of cDNA. The PCR profile was as follows: 1) denaturation at 95°C for 10 min; 2) 45 cycles of 0 s at 95°C, 5 s at 54–58°C (depending on Tm of the primers), 6 to 16 s (determined by the length of amplicon) at 72°C; 3) melting curves for 0 s at 95°C, 15 s at 65°C, and 0 s at 95°C; and 4) cooling at 40°C for 30 s. Transition rates were 20°C/s for all steps except 0.1°C/s for 95°C segment 3 of the melting curves. For data analysis, the baseline adjustment was carried out in the “arithmetic” mode, and the fluorescence analysis was carried out in the “Second Derivative Maximum” mode of the LightCycler software (version 3.5). The primers used for real-time PCR were as follows (5’ to 3’): IL-1β: sense, GGTATAAGGAGTTTGTGGCAGG and antisense, GGACATGGGAAACACCTTGTTTG; MCP-1: sense, AGCCAGATGCAATCACTGGC and antisense, CCTTGGC-CACATTGGCTTGGAA; MMP-9: sense, AGCCAGATGCAATCACTGGC and antisense, CCTTGGC-CACATTGGCTTGGAA; STAT1: sense, TTCGAGGCTCTTGGTGTTGAG and antisense, TGGGACCCAAGTGACGAGGATT and antisense, AAACGTTGATCGGTCGGGCA; and IL-6 sense, TGGTACAGGAGTGGGTGTT and antisense, CAGGAACCTGATCAAGCTGATT. Human PBMC Isolation. Venous blood was drawn from healthy donors and treated with heparin. PBMCs were isolated by density-gradient sedimentation on Ficoll-Paque (Amersham Biosciences Inc., Piscataway, NJ). PBMCs were washed twice with PBS, resuspended in RPMI 1640 cell culture medium, and then plated at 3 × 10⁶ well in 96-well plates and incubated at 37°C under 5% CO₂ for 2 h. Then, cells were pretreated with tipifarnib at 2 and 5 μM for 1 h, followed by incubation with 10 ng/ml LPS for 1 h. The supernatants were collected for cytokine assay. The groups of untreated and LPS-treated only cells were also included.

In Vivo Experiments. The experiments were performed on female BALB/c mice (6–7 weeks old; Charles River Laboratories, Inc., Wilmington, MA) after review of the protocol and approval by the Institutional Animal Care and Use Committee. Tipifarnib was dissolved in 20% cyclodextran, and 50 mg/kg was orally administered to mice at 24, 17, and 1 h before intraperitoneal injection of 20 μg of LPS per mouse, 1 mg/kg. Control mice received vehicle (20% cyclodextran) only. Blood samples were collected by eye bleeding or cardiac puncture at 2 and 3 h after LPS injection. Plasma samples were tested for cytokines using individual ELISA kits (R&D Systems) or Luminex analysis (Luminex Corporation, Austin, TX), according to the manufacturers’ instructions.

SDS-Polyacrylamide Gel Electrophoresis and Western Blot Analysis. THP-1 cells in 0.5% FBS/RPMI 1640 medium were pre-treated with or without tipifarnib for 18 h and subsequently stimulated with 100 ng/ml LPS, 10 ng/ml TNF-α, 5 ng/ml IL-1α, or 1 μg/ml Pam3CSK4 for 30 min. Cells were harvested, washed three times in ice-cold PBS, and lysed in lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na₃VO₄, 1 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Nuclear extracts were prepared using the NE-PER kit (Pierce Chemical, Rockford, IL). Cytoplasmic or nuclear lysates from each sample were mixed with an equal amount of 2× SDS-polyacrylamide gel electro-
phoresis sample buffer (Invitrogen). Proteins were separated by ele-

trophoresis in Tris-glycine 10 to 20% gradient gels (Novex, San

Diego, CA), and transferred to nitrocellulose membranes (Protr;

Schleicher & Schuell, Keene, NH). The membranes were blocked

with 3% bovine serum albumin in PBS-0.05% Tween 20 and sequen-
tially incubated with primary antibodies and horseradish peroxi-
dase-conjugated secondary antibodies (anti-rabbit IgG or anti-mouse
IgG and IgM; Pierce), followed by ECL detection (Amersham Bio-

sciences Inc.). The primary antibodies used were rabbit polyclonal

anti-IκBα, -p65, and -p38 (Santa Cruz Biotechnology, Inc., Santa

Cruz, CA), mouse monoclonal anti-H-Ras (Chemicon International,

Temecula, CA), mouse monoclonal anti-phospho-p38, rabbit polyp-
clonal anti-phospho-ERK, and anti-total ERK (Cell Signaling Tech-

nology Inc., Beverly, MA).

Results

Effect of Tipifarnib on LPS-Induced Gene Transcrip-
tion. Initial analysis of global effects for tipifarnib on tran-
scription of inflammatory genes was performed by cDNA

microarray analysis of RNA samples from THP-1 cells,
treated with LPS, in the presence or absence of tipifarnib. Of

94 genes showing at least 1.5-fold induction by LPS at two
time points, 23 showed at least a 1.5-fold decrease in LPS-
induced transcripts by tipifarnib at one or more time points
(Fig. 1). Included in the genes down-regulated by the drug
were chemokines (MCP-1 and -2), cytokines (IL-1β and
IFNβ), signaling molecules (MyD88 and STAT-1), receptors
(urokinase receptor), and proteases (MMP-9).

The results of the microarray analysis were confirmed for
selected genes by real-time PCR analysis (Fig. 2). Transcrip-
tion of IL-1β was induced by LPS as early as 3 h, and the
induction was unaffected by tipifarnib up to this time point.
However, after 6 h and, to a greater extent, after 12 h, the
LPS-dependent induction was decreased by tipifarnib (Fig.
2a). MCP-1 was induced by LPS from 6 h, and this induction
was inhibited significantly by tipifarnib (Fig. 2b). MMP-9
induction by LPS increased up to 12 h and was abolished by
treatment of tipifarnib (Fig. 2c). In the case of IL-8, induction
by LPS was evident as early as 3 h, and the FTI showed no
significant effect on this induction (Fig. 2d). STAT1 induction
was inhibited by tipifarnib at 6 and 12 h (Fig. 2e). Myeloid
differentiation factor MyD88, the adaptor for Toll-like recep-
tor-mediated responses, was significantly up-regulated by
LPS at 6 and 12 h, and this was almost completely inhibited
by tipifarnib (Fig. 2f). Although microarray data for IL-6
transcription did not meet the criteria for inclusion in Fig. 1,
analysis by quantitative real-time PCR clearly showed inhibi-
tion by tipifarnib of LPS-induced IL-6 transcription at 6
and 12 h (Fig. 2g). Thus, tipifarnib was able to inhibit LPS
induction of numerous inflammatory genes.

Effect of Tipifarnib on LPS-Induced Cytokine and
MMP-9 Production. To further investigate the effects of
tipifarnib at the protein level, cytokine and MMP-9 secretion
by THP-1 cells in response to LPS was examined. Cells were
treated with a range of concentrations of tipifarnib in the
presence and absence of LPS. Tipifarnib showed dose-depen-
dent inhibition of MMP-9 and IL-6 secretion, reaching
greater than 50% inhibition for both mediators at 2 μM (Fig.
3). This concentration was chosen to examine the effects of
tipifarnib treatment on LPS-induced inflammatory media-
tors at different times over a 48-h period. Tipifarnib showed
significant inhibition as early as 20 h for MCP-1 and IL-6 and
30 h for IL-1β and MMP-9 (Fig. 4). Tipifarnib showed no
significant inhibition of IL-8 production, consistent with the
lack of effect for tipifarnib on IL-8 transcription (Fig. 2d).
LPS-induced TNF-α also showed a trend toward reduction
after treatment by tipifarnib for 20 h (not shown), although
the production of TNF-α by THP-1 cells was low and the
inhibition did not reach statistical significance. At 2 μM,
tipifarnib had no effect on cell viability or growth up to 48 h

Fig. 1. Microarray analysis of the effect of tipifarnib on LPS-induced genes. THP-1 cells were cultured in the absence (no treatment) or presence of
100 ng/ml LPS plus or minus 5 μM tipifarnib. RNA was isolated at the indicated times for cDNA preparation as described under Materials and
Methods. Genes were selected as LPS-induced if the ratio between LPS-treated and nontreated samples was greater than 1.5-fold, at more than two
time points. Gene expression during treatment with LPS and the FTI (tipifarnib/LPS) was compared with LPS alone at the corresponding time points.
Genes that showed at least a 1.5-fold decrease in induced by tipifarnib at one or more time points were selected for inclusion in the figure. -Fold change values between LPS-treated and nontreated samples are listed and colored in orange for greater than 1.5-fold higher expression and in red for
greater than 2-fold higher expression. -Fold change values between tipifarnib/LPS and LPS are listed and colored in light blue for more than 1.5-fold
lower expression and in dark blue for more than 2-fold lower expression. The results are representative of two separate experiments.
As was also evident from the lack of inhibition of continuous IL-8 secretion.

Effect of Tipifarnib on LPS-Induced Cytokine Production in Human PBMCs. We also tested the effect of tipifarnib on primary human PBMCs. PBMCs were pre-treated with tipifarnib at 2 and 5 μM for 1 h, followed by incubation with LPS for 16 h. Tipifarnib inhibited LPS-induced TNF-α, IL-1β, and MCP-1 significantly at both 2 and 5 μM. IL-6 was significantly inhibited at 5 μM, whereas IL-8 inhibition did not reach statistical significance at either concentration (Fig. 5). Tipifarnib showed no inhibition of LPS-induced IL-10 secretion (data not shown). In the same experiment, the effect of tipifarnib at 2 and 5 μM on PBMC viability was also tested by measuring mitochondrial dehydrogenase activity, using a 2,3-bis-(2-methoxy-4-nitro-5-sulfo phenyl)-2H-tetrazolium-5-carboxanilide assay (XTT), and no significant cytotoxicity was observed (data not shown).

Effect of Tipifarnib on LPS-Induced Cytokine Production in Vivo. To follow-up on our in vitro observations, we tested the effect of tipifarnib pretreatment in a murine model of LPS-induced inflammation. The mice were pretreated for 24 h with the compound by oral administration...
at three time points: 24, 17, and 1 h before LPS injection. Blood samples were taken 2 and 3 h after LPS injection for analysis.

As shown in Fig. 6, the most striking effect of the FTI was its highly significant inhibition of LPS-induced TNF-α production at 2 h. Approximately 50% inhibition of IL-6 production, and almost complete inhibition of IL-1β production, was observed in tipifarnib-treated animals after 3 h. Approximately 50% inhibition of MIP-1α and MCP-1 production by the FTI was observed at 2 h. IL-12-p40 and -p70 induction by LPS was also inhibited by tipifarnib at 3 h, whereas IL-10 was not significantly changed at both time points (data not shown). No effects of tipifarnib on LPS-induced KC were observed, consistent with in vitro results for IL-8.

**Effect of Tipifarnib on TLR/IL-1R Ligand and TNF-α-Induced Signaling.** It was recently reported that TNF-α-induced NF-κB activity was inhibited by another FTI (SCH 66336) (Takada et al., 2004). To compare the effects of tipifarnib to this finding, we tested TNF-α-induced NF-κB activation by luciferase reporter activity in human embryonic kidney 293 cells. Cells were treated with tipifarnib overnight, followed by stimulation with TNF-α for 4 h. In contrast to the results reported for SCH 66336, TNF-α-induced NF-κB activity was not inhibited by tipifarnib at 2 or 5 μM (data not shown). We further examined the effect of tipifarnib on several signaling pathways in THP-1 cells. Cells were pretreated with 2 μM tipifarnib for 18 h and then stimulated with LPS or TNF-α for 30 min. Cell lysates were tested for p38 expression and p38 phosphorylation by Western blot analysis. Phosphorylation of p38 was significantly increased by LPS and TNF-α stimulation, but only the LPS-induced increase was partially reduced by tipifarnib treatment (Fig. 7a). No significant inhibition was observed for TNF-α-induced p38 phosphorylation. p38 protein expression was not affected by LPS or TNF-α stimulation and/or tipifarnib treatment.

As shown in Fig. 7, b and c (top), IκB-α degradation was significantly induced by TLR/IL-1R ligands (LPS, Pam3CSK4, and IL-1α) and TNF-α. Interestingly, tipifarnib significantly inhibited LPS-induced IκB-α degradation (Fig. 7b, lane 4; c, lane 6) but showed no inhibition of IκB degradation induced by TNF-α or IL-1α (Fig. 7b) or by Pam3CSK4 (Fig. 7c). We further determined the effect of tipifarnib on NF-κB translocation by immunoblotting of p65 in nuclear extracts. Tipifarnib almost completely suppressed nuclear translocation of p65 induced by LPS but not that induced by TNF-α and Pam3CSK4 (Fig. 7c, bottom).

The level of ERK phosphorylation was similar in cells...
treated with different stimuli, and tipifarnib pretreatment reduced the ERK phosphorylation to a similar extent under all conditions, without affecting the level of total ERK protein (Fig. 7b, middle). We also examined the effect of tipifarnib on H-Ras farnesylation (Fig. 7b, bottom). The level of farnesylated (or processed) H-Ras was not significantly changed in the absence or presence of inflammatory stimuli. Tipifarnib partially inhibited H-Ras farnesylation, as shown by the presence of unprocessed Ras, to the same degree in the different treatment groups, and this inhibition correlated with the extent of inhibition of ERK phosphorylation (Fig. 7b, second panel).

**Discussion**

In the present study, we investigated the anti-inflammatory activity of the PTI, tipifarnib, both in vitro and in vivo. Tipifarnib demonstrated inhibition of both transcription and secretion of IL-6, IL-1β, MCP-1, and MMP-9 induced by LPS treatment of THP-1 cells as well as dose-dependent inhibition of LPS-induced TNF-α, MCP-1, IL-1β, and IL-6 production in primary human PBMCs, without any evidence for cytotoxicity. We also observed inhibition by tipifarnib in THP-1 cells of LPS-induced Myd88 and STAT1 transcription, providing the first evidence for a role of farnesyltransferase in regulation of these genes. Global anti-inflammatory effects of tipifarnib were also observed in vivo, including down-regulation of LPS-induced TNF-α, IL-6, MCP-1, IL-1β, and MIP-1α.

Tipifarnib had contrasting effects between LPS-induced IL-8 and other mediators both in THP-1 and human PBMCs. No inhibition of LPS-induced IL-8 transcription or secretion was observed in our studies for tipifarnib, at concentrations that were highly effective at inhibiting IL-1β, IL-6, and MCP-1 production. Consistent with the in vitro data, no inhibition of production of the murine homolog KC was observed in vivo. The results suggest that the target(s) of tipifarnib may be less crucial for optimal expression of IL-8 than for that of other cytokines or chemokines.

A recent study has shown inhibition in vitro and in vivo of LPS-induced nitric oxide, prostaglandin E₂, TNF-α, and IL-1β by the FTI LB42708 (Na et al., 2004). Our present study represents the first observation of FTI-mediated inhibition of LPS-induced IL-6, MCP-1, and MIP-1α in vivo. We also observed inhibition in vivo by tipifarnib of LPS-induced IL12-p40 and -p70. Although Na et al. (2004) showed inhibition by LB42708 of TNF-α and IL-1β production in vivo, the LPS-induced cytokine levels were 10- to 100-fold lower than we obtained. The cytokines were measured 12 h after LPS administration in their study (Na et al., 2004), which is long after the peak between 1 and 3 h for LPS-induced TNFα and IL-1β in this model. We also investigated the effect of tipifarnib on IL-10, which is known as an anti-inflammatory mediator, and have shown that tipifarnib had no effect on LPS-induced IL-10 production, both in human PBMCs and in vivo.

Inhibition of inflammatory mediators by tipifarnib seemed to require pretreatment time. For example, cytokines such as IL-1β showed significant transcriptional induction by LPS as early as 3 h in vitro. When drug treatment was started at the same time as the LPS stimulation, the earliest signs of transcriptional inhibition were only observed after 6 h and of cytokine release only after 22 h. In contrast, when animals were pretreated with the drug for 24 h before LPS challenge in vivo, inhibition of cytokine release could be observed as early as 1 h after the challenge (data not shown). The lag time in the inhibition of inflammatory responses may indicate a requirement to deplete intracellular pools of particular prenylated proteins.

Previous animal studies with tipifarnib have shown inhibition of cachexia in a tumor xenograft model and reduction of disease scores in dextran sodium sulfate-induced colitis and collagen-induced arthritis, but the mechanism for these effects was unknown (David End, unpublished observations). The major roles of TNF-α, IL-6, and IL-1β in the pathogenesis of these diseases suggest that their inhibition by tipifarnib may have been responsible for the observed amelioration. Although we did not measure the plasma concentrations of tipifarnib at which we observed inhibition of inflammatory mediators in vivo, the doses where we observed anti-inflammatory effects in the LPS-induced inflammation model were similar to those used previously for tumoricidal effects in mouse xenograft studies (End et al., 2001).

It is of interest to note that in phase I studies with tipifarnib (300–900 mg/day) in myelodysplastic syndrome, the only correlation with positive response to treatment was a...
42% median decrease in serum levels of TNF-α (Kurzrock et al., 2003). Tipifarnib, 300 mg twice a day, was the maximum tolerated dose in a number of studies (Head and Johnston, 2003). At this dose, plasma levels reached approximately 2 μM (Karp et al., 2001), at which concentration significant inhibition of inflammation was obtained in vitro in our present study. Thus, sufficient plasma concentrations of tipifarnib for anti-inflammatory effects were reached in clinical trials where positive responses were observed, but existing data are insufficient to allow any direct correlation.

To explore the underlying mechanism for the anti-inflammatory activity of tipifarnib, we examined the effect of tipifarnib for anti-inflammatory effects were reached in clinical trials where positive responses were observed, but existing data are insufficient to allow any direct correlation. We found that inhibition of TNF-α mediated signaling by tipifarnib was clearly evident. Tipifarnib, 300 mg twice a day, was the maximum tolerated dose in a number of studies (Head and Johnston, 2003). At this dose, plasma levels reached approximately 2 μM (Karp et al., 2001), at which concentration significant inhibition of inflammation was obtained in vitro in our present study. Thus, sufficient plasma concentrations of tipifarnib for anti-inflammatory effects were reached in clinical trials where positive responses were observed, but existing data are insufficient to allow any direct correlation. The Ras/Raf/mitogen-activated protein kinase pathway has shown that Ras controls TRAF6-dependent induction of NF-κB (Caunt et al., 2001). We found that inhibition of Ras farnesylation, both in the absence and in the presence of inflammatory stimuli. These data confirm that tipifarnib suppresses ERK1/2 phosphorylation through inhibition of Ras farnesylation.

LPS, Pam3CSK4, IL-1, and TNF-α each interact with their own receptors to trigger the corresponding inflammatory signaling cascades (Fig. 8). TNF-α signaling links to IKK through TNFR/receptor interacting protein (Aggarwal, 2003). LPS/TLR4, Pam3CSK4/TLR2, and IL-1/IL-1R signaling all link to IKK through the shared MyD88-dependent pathway (Akira and Takeda, 2004). IKK phosphorylates IκBα, which leads to the ubiquitination and degradation of IκB, and thus enables the translocation of NF-κB to the nucleus and induction of target gene expression such as TNF-α, IL-1β, IL-6, and others. Besides the common MyD88-dependent pathway shared with IL-1R and TLR2, the interaction of LPS and TNF-α also induces IκB degradation through MyD88-independent pathways, e.g., through TRIF domain-containing adapter protein inducing IFN-β (TRIF) and TRIF-related adapter molecule (TRAM) (Akira and Takeda, 2004). We found that tipifarnib inhibited IκB-α degradation induced by LPS, but not by TNF-α, Pam3CSK4, or IL-1, suggesting that the target protein for tipifarnib is not involved in TLR2/IL-1R and TNFR signal pathways, but it is involved in a pathway exclusive to LPS/TLR4 signaling.

A recent study has shown that Ras is involved in CpG oligonucleotide signaling as an early event, by associating with TLR9 and promoting IL-1 receptor-associated kinase (IRAK)/TRAF6/transforming growth factor-β-activated kinase (TAK) pathway (Akira and Takeda, 2004). IKK phosphorylates IκBα, which leads to the ubiquitination and degradation of IκB, and thus enables the translocation of NF-κB to the nucleus and induction of target gene expression such as TNF-α, IL-1β, IL-6, and others. Besides the common MyD88-dependent pathway shared with IL-1R and TLR2, the interaction of LPS and TNF-α also induces IκB degradation through MyD88-independent pathways, e.g., through TRIF domain-containing adapter protein inducing IFN-β (TRIF) and TRIF-related adapter molecule (TRAM) (Akira and Takeda, 2004). We found that tipifarnib inhibited IκB-α degradation induced by LPS, but not by TNF-α, Pam3CSK4, or IL-1, suggesting that the target protein for tipifarnib is not involved in TLR2/IL-1R and TNFR signal pathways, but it is involved in a pathway exclusive to LPS/TLR4 signaling.
Fig. 8. Proposed target(s) for tipifarnib inhibition of LPS-induced inflammatory signaling pathways. Signals from TLR4/IL-R through the MyD88-dependent pathway or from TNFR1 through receptor interacting protein/MEKK induce the activation of the IKK complex. IKK phosphorylates IκB, inducing its degradation, which enables translocation of NF-κB to the nucleus and induction of target gene expression. LPS binding to TLR4 also induces activation of NF-κB through MyD88-independent pathways. The data in the present study suggest that the target for anti-inflammatory effects of tipifarnib is a component of pathways exclusive to LPS/TLR4 signaling, as indicated in bold (TLR2-L, TLR3 ligands).

1α- and TNF-α-treated cells, but inhibition of IκB-α degradation and NF-κB translocation was only observed in the LPS-treated cells. This lack of correlation between inhibition of Ras farnesylation and inhibition of NF-κB signaling indicates that Ras is unlikely to be the target protein mediating the anti-inflammatory effects of tipifarnib. Candidate targets for tipifarnib could be the function or expression of TLR4 itself, or associated proteins such as CD14 or myeloid differentiation protein-2, or other molecules unique to the LPS signaling pathway, either upstream or independent of MyD88.

In conclusion, we present the first evidence for anti-inflammatory effects of the FTI tipifarnib and expand and highlight the potential for farnesyltransferase inhibition as a therapeutic approach to inflammation. We show inhibition by tipifarnib of a number of LPS-induced genes not previously known to be dependent on protein farnesylation. In particular, tipifarnib inhibited LPS induction of MCP-1, IL-6, MMP-9, MIP-1α, STAT1, and MyD88 in addition to TNF-α and IL-1β, all of which have been implicated in inflammatory diseases. Our data suggest that the target protein for these effects of tipifarnib is likely to be a component of the TLR4 pathway, upstream or independent of MyD88, and is unlikely to be Ras, in contrast to the conclusions of Na et al. (2004) for the FTI LB42708. Elucidation of the identity of the target protein or proteins may provide new targets for anti-inflammatory therapy. Furthermore, some of the therapeutic efficacy of FTIs in cancer may be related to inhibition of inflammatory mediators, which have been shown to contribute to cancer progression.

References
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