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Review Article

Thalidomide as a blocker of TNF production

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CONTENTS

Introduction

Thalidomide (Fig. 1), first synthesized as an antihistaminic in 1954, was introduced as a hypnotic in 1956. Its main advantage over other sleep-inducing drugs available at the time was its lack of acute side effects. The acute toxicity was so low that a standard lethal dose in mice could not even be determined. In humans, even with high doses, there was no respiratory depression or mortality (1). Moreover, as a powerful antiemetic, the drug was used to treat nausea associated with first trimester morning sickness in pregnant women. Due to the initial enthusiasm over low toxicity, the drug was tested for a variety of indications, including psychosis, hyperthyroidism and asthma (1). One study also found an analgesic effect, especially in postoperative patients (2). However, the drug was taken off the market in 1961 because of its catastrophic teratogenicity (3, 4). Another side effect observed with long-term treatment was the occurrence of an axonal, sensorimotor neuropathy (5, 6).

In the early sixties, thalidomide was used as a sedative in patients with lepromatous leprosy (erythema nodosum leprosum, ENL). The rapid and thorough improvement of painful neuritis experienced by these patients was a serendipitous discovery which was published in 1965 (7). After a series of controlled studies on the subject, thalidomide became a standard treatment for ENL (8). However, the mechanism of action was unknown for a long time. After Sarno *et al.* found increased levels of tumor necrosis factor (TNF)-α and interleukin-1β (IL-1β) in the serum of patients with ENL (9), the possibility of a TNF inhibiting action of thalidomide was investigated. Indeed, a selective inhibition of TNF production by stimulated macrophages in culture could be shown

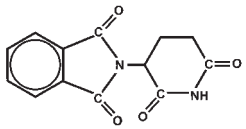

Fig. 1. Structure of thalidomide (α-phthalimidoglutarimide).

(10), as well as a reduction of elevated TNF levels in the serum of ENL patients (11). Since then, thalidomide has been used as therapy for a variety of diseases associated with elevated TNF, with variable success (12). In addition, the immunological actions of thalidomide have been investigated by various groups. Some of these effects may be secondary to TNF blockade and others may be independent (13).

TNF blockade *in vitro*

Sampaio *et al.* were the first to show inhibition of TNF production by stimulated macrophages in culture (10). As assessed by ELISA, thalidomide suppressed lipopolysaccharide (LPS)-stimulated TNF production with a 50% inhibitory concentration of 1-4 μg/ml; a 90% inhibition was observed at a dose of 10 μg/ml. A 40% inhibition occurred at a concentration of 1 μg/ml, a clinically achievable dose. The effect was not due to inhibition of protein synthesis, since total protein synthesis was not changed by thalidomide. Also, the same dose that inhibited TNF synthesis did not alter the levels of IL-1β, IL-6 or granulocyte-macrophage colony-stimulating factor (GM-CSF). In these assays, the timing of treatment was important in that inhibition only occurred after the induction of TNF production by the stimulus. Pretreatment of the cells was not effective. The reduction of TNF synthesis in macrophages by thalidomide was confirmed by other investigators (14), as well as the reduction of TNF synthesis in alveolar macrophages (15). In primary human fetal microglial cell cultures stimulated with LPS or lipoarabinomannan, thalidomide also inhibited TNF release in a dose-dependent manner (16). Thalidomide reduced TNF production in stimulated HIV-1 infected U1 cells, a promonocytic line, whereas the same treatment did not reduce TNF levels in the T cell line ACH-2 (17).

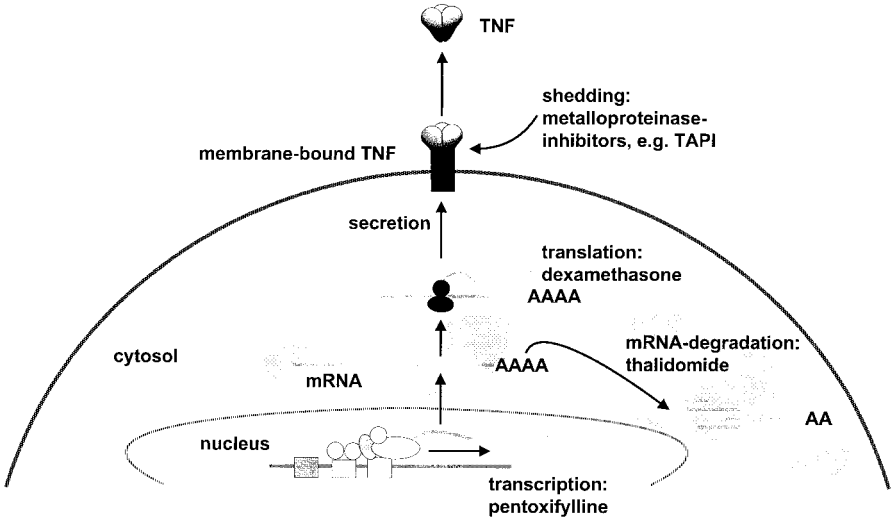

Fig. 2. Targets of several TNF blocking drugs. Thalidomide presumably reduces TNF by enhancing TNF mRNA-degradation (modified from ref. 13.)

In contrast, in some human leukemia cell lines, thalidomide was shown to enhance 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced TNF production (18, 19). If the same cells (in this case HL-60 cells) were stimulated with okadaic acid (OA), thalidomide inhibited TNF production. Thus, in addition to a cell-specific effect, an inducer-specific bidirectional regulation of TNF production could be observed. In another human leukemia cell line, TP-1 cells, thalidomide inhibited TNF production after stimulation with either TPA or OA (20). Moreover, in cultures of LPS-stimulated human mononuclear cells enriched for adherent cells and in cultures of LPS-stimulated human monocytes of the cell line THP-1, thalidomide slightly enhanced the synthesis of TNF. When cultures of unfractionated peripheral blood mononuclear cells (PBMC) were stimulated with LPS, thalidomide decreased the synthesis of TNF. Thus, depending on the type of cells stimulated with LPS *in vitro*, thalidomide could either enhance or suppress TNF synthesis (21). One possible explanation for this bidirectional effect may be the existence of different target molecules for thalidomide on different cell types, which mediate either the enhancing or the inhibiting effect (22).

Regarding the mechanism of TNF inhibition, Moreira *et al.* demonstrated that thalidomide enhanced the degradation of TNF mRNA. The half-life of the molecule was reduced from approximately 30 to 17 min in the presence of 50 μg/ml of thalidomide (23). This was in contrast to other TNF inhibitors such as pentoxifylline, which reduces TNF transcription, and dexamethasone, which impedes translational depression (24, 25) (Fig. 2). These results could not be entirely reproduced in a different *in vivo* model (13). Here, stimulation of mice with *Staphylococcus aureus* enterotoxin B was used and splenic mRNA was analyzed. Thalidomide did reduce splenic TNF mRNA, but not sufficiently to explain the marked effect on serum concentrations of TNF protein. The exact molecular mechanism of the thalidomide action is still unknown. α_1-Acid glycoprotein (AGP) has been shown to be a molecular target of the drug (26). AGP is an acute-phase protein, which has been shown to provide protection against TNF-induced lethality, although it also has been shown to enhance LPS-induced TNF production (27).

TNF blockade *in vivo* in animals

In vivo experiments mostly confirmed the inhibitory action of thalidomide on TNF production. The constitutively elevated TNF level in magnesium deficient mice was reduced by a dose of 1 mg/day of thalidomide, whereas the increased levels of IL-1 were not affected (28). In tumor bearing mice stimulated with 5,6-dimethylxanthenone-4-acetic acid, thalidomide reduced serum TNF levels by 80% (29).

Burroughs *et al.* investigated the ability of thalidomide to modulate the inflammatory response to either lysates of the Gram-negative bacteria *H. influenzae* or heat killed Gram-positive *S. pneumoniae* in rabbits. *H. influenzae* lysate in the CSF of rabbits caused a very acute inflammatory response with a rapid increase in TNF levels in the CSF. Heat killed *S. pneumoniae* induced a more indolent inflammatory response. Thalidomide treatment reduced TNF production in both experimental systems, but had a greater effect on the more indolent Gram-positive inflammatory response (50% reduction of TNF *vs.* 29%). IL-1 levels were not significantly altered (30). Netea *et al.* studied mice infected with *Candida albicans*. Here, serum TNF levels were significantly reduced after thalidomide treatment (31).

In mice infected with *Mycobacterium tuberculosis*, which induced high serum and lung cytokine mRNA

levels, thalidomide treatment (30 mg/kg/day) resulted in a significant reduction in TNF, IL-6 and IL-10 protein levels in the serum and a reduction of their mRNA expression in the lung; IL-12 and interferon-γ (IFN-γ) were unaffected. A treatment effect observed was that the lungs of thalidomide-treated mice had smaller granulomata with apoptotic cells and no necrosis (32). The same group showed dramatically reduced serum TNF levels in mice with LPS-induced shock. Thalidomide treatment (200 mg/kg) also enhanced survival at otherwise lethal doses of LPS (33). Similar results have been previously reported by Schmidt *et al.* in LPS-treated rats (34). In contrast, Ishikawa *et al.* reported that thalidomide administered in very low doses (1-6 mg/kg) promoted the release of TNF and lethality after LPS-stimulation in mice (35). In a rabbit model of severe tuberculous meningitis with vasculitis and encephalitis, the combination of antituberculous therapy with thalidomide reduced TNF levels and brain pathology with 100% of the infected rabbits surviving as compared to only 50% in the group receiving antibiotics alone (36).

A different *in vivo* model was used by our group. Considering the important role TNF plays in inflammatory pain (37) and that temporal concordance of hyperalgesia occurs after nerve lesions accompanied by an increase in TNF in the lesioned nerve (38), we postulated that reduction of TNF levels might alleviate neuropathic pain. We used thalidomide (50 mg/kg by gavage) in rats with a painful mononeuropathy and showed a reduction of thermal hyperalgesia and mechanical allodynia in treated animals (39). TNF was reduced in the damaged nerves treated with thalidomide when compared with vehicle treatment, as shown by immunohistochemistry and ELISA (39, George and Sommer, unpublished).

Other investigators have described therapeutic effects of thalidomide in inflammatory conditions that were apparently not TNF-dependent. For example, Olier *et al.* found a positive effect of thalidomide (200 mg/kg b.i.d.) in collagen-induced arthritis in rats without changes in serum TNF. It is unclear, however, whether local TNF concentrations in inflamed tissue were changed (40).

TNF blockade in humans

The first reports of *in vivo* blockade of TNF production in humans came from studies involving patients with leprosy, where thalidomide was given for the treatment of erythema *Nodosum leprosum* (ENL). Barnes *et al.* showed that TNF secretion of stimulated PBMC from patients with ENL was much higher than from patients with tuberculoid leprosy. Thalidomide treatment of patients with ENL reduced TNF production from their PBMC by 90% (11). However, in this study, TNF serum levels were not reported. Circulating TNF levels were measured by ELISA by Sampaio *et al.*, who showed high levels in ENL patients and prompt reduction of these when thalidomide was administered (41, 42). Their results were confirmed in a group of 9 Mexican patients (43).

In 30 patients with active tuberculosis, thalidomide reduced TNF production *in vivo* and *in vitro* (44). Similar results were also observed in patients with HIV and tuberculosis infection where TNF levels were found to be lower in the thalidomide-treated group than in the placebo group (45). However, in the same study, thalidomide did not change TNF levels in patients with HIV only. Huizinga *et al.* reported decreased production of TNF by LPS-stimulated *ex vivo* whole blood cultures of thalidomide-treated patients with rheumatoid arthritis with no effect on IL-6, IL-10 and IL-12 (46). No significant changes in the serum levels of TNF and TNF receptors were found in 19 asymptomatic HIV-positive patients (47). Jacobson *et al.* found increases in plasma concentrations of TNF and soluble TNF receptors in thalidomide treated HIV-positive patients with oral aphthous ulcers (48).

Treatment of diseases with thalidomide

As mentioned above, leprosy patients with ENL were the first individuals who apparently profited from thalidomide-induced reductions in TNF (7). Thalidomide became a standard treatment for ENL long before its mechanism of action in this disease was known (49). Also, positive effects in many other inflammatory conditions have been reported which only later were partly attributed to TNF suppression (8). After reduction of elevated TNF was recognized as the mechanism of action in ENL (11, 41, 42), the use of thalidomide was extended as a therapy for other diseases in which elevated TNF levels were observed to be one of the pathogenically important factors.

The first report on successful use of thalidomide in aphthous ulcers was by Mascaro *et al.* (50). Later, a multicenter, randomized, placebo-controlled, crossover trial was conducted in which thalidomide 100 mg was given for 2 months to 73 patients with severe aphthous stomatitis of more than 6 months duration. Complete remission was obtained in 44% of the thalidomide-treated patients. Most of the thalidomide patients who did not achieve a complete remission showed dramatic improvements (51). Multiple case reports followed on thalidomide in aphthous ulcers in HIV-positive patients and ulcers in Behçet's disease (12). A controlled study was conducted by Jacobson *et al.* (48). The series of 59 patients with oral or genital ulcers reported by Gardner-Medwin *et al.* (52) included 23 patients with Behçet's disease. A dose of 200 mg/day was sufficient to cause complete resolution of the ulcers in 81% of patients. In most patients, maintenance therapy was needed, although at lower doses. Dramatic improvements were also reported for colitis in Behçet's disease (53). In a randomized, double-blind, placebo-controlled trial, however, only 2/32 patients with orogenital ulcers responded to thalidomide 100 mg/day and 5/31 patients to 300 mg/day. This response, was however, significantly better than that observed with placebo (0/32 patients) (54).

TNF is one of the factors responsible for the development of graft *versus* host disease (GVHD) (55). After successful animal experiments with thalidomide in GVHD (56), initial case reports gave favorable results in treated patients (57-64). A small study suggested a beneficial effect of thalidomide for the chronic rather than the acute form of GVHD (65). Heney *et al.* found thalidomide to be beneficial in 5/6 patients with chronic GVHD (66) and Volgelsang *et al.* reported 30/44 patients, including high-risk patients (67). Among the 80 patients studied by Parker *et al.*, only 16 had a sustained response to thalidomide and 29 had the drug discontinued due to side effects (68). Children seem to have a more favorable response (69, 70).

Since wasting and cachexia are TNF-mediated, thalidomide (400 mg/day) was given in the HIV-associated wasting syndrome. In a double-blind, placebo-controlled study, 8/14 patients from the verum group gained weight compared to only 1 patient in the placebo group. Thalidomide had no effect on CD4 T cell counts or HIV viral burden in PBMC (71). In the study conducted by Klausner *et al.*, 32 patients received either thalidomide or placebo (45). In patients with concomitant HIV-1 and tuberculosis infections, thalidomide therapy was associated with a reduction in both plasma TNF and HIV-1 levels, whereas no significant reduction in either level was observed in patients with HIV-1 infection only. Patients receiving thalidomide treatment showed significant weight gain. Again, patients with simultaneous HIV-1 and tuberculosis infections experienced a higher mean weight gain than patients with HIV-1 infection alone. Tramontana *et al.* investigated the effect of thalidomide in 30 patients with active tuberculosis, who were either HIV-positive or negative. A favorable effect on weight was observed in both groups, while TNF production was reduced and IFN-γ production enhanced (44).

Thalidomide was used in a variety of other HIV-associated disease states. Two patients with HIV-associated painful proctitis responded rapidly to 300 mg/day of thalidomide (72). Of 12 patients with HIV-associated esophageal ulcers, 11 responded very well to a dose of 200 mg/day of thalidomide (73). Jacobson *et al.* found thalidomide (200 mg/day *vs.* placebo) to be an effective treatment for HIV-positive patients with oral aphthous ulcers, although plasma concentrations of TNF and soluble TNF receptors were unexpectedly increased (48). In a study reported by Sharpstone *et al.*, thalidomide was given to HIV-positive patients with microsporidiosis with the rationale that fecal TNF is elevated with this infection. The response was good to excellent in 10/18 patients, while fecal TNF levels were nonsignificantly decreased (74).

In 1989, an open study in 17 patients with refractory rheumatoid arthritis reported encouraging results so that controlled studies with thalidomide in this indication were warranted (75). In contrast, Huizinga *et al.* found no benefit of thalidomide given as an adjuvant therapy in patients with rheumatoid arthritis, although the drug reduced production of TNF by LPS-stimulated *ex vivo* whole blood cultures (46).

In 29 patients with uremic pruritis refractory to other forms of therapy, 55% had a mean reduction in pruritis scoring of 78% after thalidomide treatment (76). Also, patients with prurigo nodularis had a good response to combined treatment with thalidomide and ultraviolet B therapy (77). A variety of other dermatological conditions have been treated with thalidomide, such as bullous pemphigoid, psoriasis or allergic cutaneous vasculitis and pyoderma gangrenosum (12, 78, 79). Of 16 patients with cutaneous lupus, 7 had remission of skin disease and 6 had partial remission with doses of 50-100 mg/day (80). In Langerhans granulomatosis, which is also associated with TNF upregulation, treatment with thalidomide was successful according to several case reports (81-83).

Bessis *et al.* reported a good response for dermal and arthricular symptoms in 3 patients with systemic lupus erythromatosus (SLE) who were administered thalidomide (100 mg/day) as an add-on therapy (84). Out of 23 treated SLE patients, 90% had remission of cutaneous and arthricular symptoms while visceral symptoms remained unchanged (85). Additionally, various case reports suggest beneficial actions of thalidomide in other inflammatory conditions, such as Melkersson-Rosenthal syndrome (86), Crohn's disease (87) and sarcoidosis (88, 89).

As is obvious from these numerous clinical studies and case reports (Table I), in most instances, definite proof that the effect of thalidomide is mediated through TNF blockade is not given. In some cases, a treatment effect was achieved even though TNF levels were increased. However, modulation of TNF levels may play a role in the treatment of these diseases and syndromes, and therefore further investigation of the exact mechanisms of action of thalidomide could lead to new insights into the pathogenesis of the disorders.

Side effects

There is obvious consensus that, due to the devastating teratogenic effects of the drug, thalidomide should only be given to male patients and women not of childbearing age. If under special circumstances the drug is administered to women of childbearing age, strict birth control must be practiced and enforced. The mechanism by which the embryopathic effect occurs is still unknown, although various hypotheses have been offered (90). One of the more recent ones is that the teratogenic effect is due to blockade of TNF during development (91). However, the evidence supporting this is not convincing. Another concern is thalidomide neuropathy, an adverse effect first discovered when the drug was still on the market as a hypnotic (5, 6). Clinically, the neuropathy is characterized by symmetric painful paresthesia and distal hypesthesia of the legs. Electrophysiology is consistent with an axonal neuropathy which was also confirmed in sural nerve biopsies (92, 93). Interestingly, the incidence of thalidomide neuropathy seems to be quite variable; it was found in < 1% of patients with ENL (49) and in > 70%

Table I: Studies and selected case reports of thalidomide treatment in disorders presumably associated with an increase in TNF.

Author/Date	Disorder	Type of Study/No. of patients	Effect of treament	Effect on TNF
Barnes 1992	ENL	Open/ND	ND	↓ *Ex vivo*
Sampaio 1992	ENL	Open/10	++	↓ *In vivo*
Sampaio 1993	ENL	Open/24	++	↓ *In vivo*
Partida-Sanchez 1998	ENL	Open/9	++	↓ *In vivo*
Gutierrez 1989	RA	Open/17	+	ND
Huizinga 1996	RA	Open/12	–	↓ *Ex vivo*
McCarthy 1989	GVHD	Open/6	++ in chronic form – in acute form	ND
Heney 1991	GVHD	Open/6	++ in 4, + in 1	ND
Vogelsang 1992	GVHD	Open/44	++ in 30	ND
Cole 1994	GVHD	Open/5 children	++	ND
Parker 1995	GVHD	Open/80	+ in 16	ND
Rovelli 1998	GvHD	Open/14 children	+ in 10 of 14	ND
Tramontana 1997	TB (+HIV)	Placebo cont./30	++	↓ *In vivo*, *in vitro*
Klausner 1996	HIV (+TB)	DBRPC/32	++	↓ *In vivo* in TB
Georghiou 1992	HIV proctitis	Open/2	++	ND
Reyes-Teran 1996	HIV wasting	DBRPC/28	++ in 11 of 14	ND
Alexander 1997	HIV esoph. ulcers	Open/12	++ in 11	ND
Jacobson 1997	HIV aphth. ulcers	DBRPC/57	++	↑ *In vivo*
Sharpstone 1997	HIV microspor.	Open/18	++ in 10	↓ In stool
Silva 1994	Uremic pruritus	DBRPC/29	++ in 55%	ND
Gardner-Medwin 1994	Behçet and other ulcers	Open/59	++ in 81%	ND
Postema 1996	Behçet colitis	Open/1	++	ND
Hamuryudan 1998	Behçet ulcers	DBRPC/96	++ in 7 of 63	ND
Revuz 1990	Aphth. stomatitis	DBRPC/73	++ in 32	ND
Bensaid 1992	LH	Open/1	++	ND
Thomas 1993	LH	Open/2	++	ND
Dallafior 1993	LH	Open/1	++	ND
Ferrandiz 1997	Prurigo nodularis	Open/4	++	ND
Stevens 1997	Cutaneous LE	Open/16	+-++ in 13	ND
Bessis 1992	SLE	Open/3	++ (skin and arthricular sympt.)	ND
Atra 1992	SLE	Open/23	++ in 18 (skin)	ND

ENL: erythema nodosum leprosum. RA: rheumatoid arthritis. GVHD: graft *versus* host disease. TB: tuberculosis. HIV: human immunodeficiency virus infection. LH: Langerhans histiocytosis. LE: lupus erythematosus. SLE: systemic lupus erythematosus. DBRPC: double-blind, randomized, placebo-controlled trial. Treatment effect: ++ very good, + good, – poor. ND: not determined.

of patients treated for prurigo nodularis (94). Monitoring by electrophysiology is advisable (52, 95) since the neuropathy, once established, tends to persist even after discontinuation of the drug (92). Other frequently observed side effects include drowsiness, dizziness, mood changes, constipation and dry mouth (12).

New thalidomide derivatives

Efforts have been made to develop derivatives of thalidomide that would specifically maintain the desired actions of the drug without its side effects. One approach was to separate the effects of the (*R*)-enantiomer and the L-enantiomer of the drug. Since racemization of thalidomide is very fast, no difference between the biological activities of the enantiomers under physiological conditions were observed (96-98). Using stable nonracemizable analogs of thalidomide, *e.g.*, α-methylthalidomide (Fig. 3), the (*R*)-isomer was shown to be a potent inhibitor of TNF production in certain cell lines, while the L-isomer was a TNF enhancer in certain systems (20, 96). In

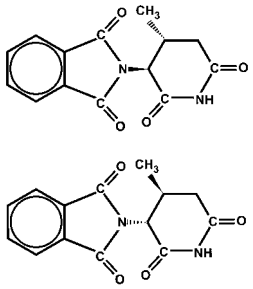

Fig. 3. Structures of the *S*-isomer (top) and *R*-isomer (bottom) of α-methylthalidomide.

human PBMC stimulated with LPS, the (*S*)-isomer was the more potent inhibitor (99). In addition, a wide variety of thalidomide related compounds, mostly *N*-phenylphthalimide and *N*-benzylphthalimide derivatives have been tested for their TNF production regulating activities, mostly with the purpose of identifying compounds with a strong TNF inhibition and lack of teratogenicity (100). Some of these compounds were found to have multiple

pharmacologic activities and further research seems promising (22, 101).

Conclusions

There is no doubt that thalidomide and some of its analogs block TNF production in certain settings. However, depending on the experimental and biological conditions, there may be no effect on TNF levels or an enhancement in TNF production may be observed. Results from *in vitro* studies demonstrate that the effect of thalidomide, whether inhibitory or enhancing, depends on the cell line as well as on the stimulus used to induce TNF synthesis. Constitutive TNF production, in general, seems not to be blocked. *In vitro* studies have mostly been conducted using PBMC or human leukemia cell lines. These observations make it difficult to extrapolate the *in vivo* situation. Moreover, thalidomide action *in vivo* is also often unclear since the exact target TNF-producing cell is usually unknown. For example, macrophages and T cells may behave differently in HIV-associated disorders. Also, very little is known about the effect of thalidomide on TNF production by glial cells and Schwann cells.

Of the many disease states in which thalidomide is now being used with the intention of blocking TNF, in some with considerable clinical success, only a few studies really demonstrate TNF reduction *in vivo*. Further research is warranted to determine the mechanism of thalidomide action in these disorders. This knowledge may lead to the further development of more selective and potent thalidomide analogs.

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