Thymidine Dinucleotides Inhibit Contact Hypersensitivity and Activate the Gene for Tumor Necrosis Factor α[1]

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DNA is a target for ultraviolet-B-induced inhibition of contact hypersensitivity, and small DNA fragments such as thymidine dinucleotides (pTpT) can simulate several ultraviolet-induced effects. To determine whether pTpT mimics the suppressive influence of ultraviolet-B on contact hypersensitivity, we compared the effects of topical application of pTpT with those of ultraviolet-B irradiation on C57BL/6 mice sensitized to dinitrofluorobenzene. Mice pretreated with pTpT or ultraviolet-B irradiation showed markedly suppressed ear swelling responses to dinitrofluorobenzene challenge. Because tumor necrosis factor α mediates ultraviolet-B-induced suppression of contact hypersensitivity, and because pTpT exerts many ultraviolet-mimetic effects by augmenting mRNA and protein levels of effector molecules, we asked if pTpT mimics ultraviolet-B's upregulatory influence on tumor necrosis factor α expression. Using transgenic mice carrying a chloramphenicol acetyl transferase reporter linked to the tumor necrosis factor α promoter, we examined effects of ultraviolet-B irradiation *versus* intradermal injection of pTpT on tumor necrosis factor α gene transcription. Both treatments induced cutaneous chloramphenicol acetyl transferase activity. Ultraviolet-B or pTpT treatment of cultured dermal fibroblasts from these mice also stimulated chloramphenicol acetyl transferase activity. To determine whether human cells responded similarly, a well-differentiated ultraviolet-responsive human squamous cell carcinoma line was treated with pTpT. pTpT increased tumor necrosis factor α mRNA expression and protein secretion in a dose-dependent manner. Our findings expand the spectrum of ultraviolet effects mimicked by pTpT to include inhibition of contact hypersensitivity and activation of the tumor necrosis factor α gene. These results support the hypothesis that DNA photoproducts and/or their repair intermediates trigger many of the biologic consequences of ultraviolet irradiation.
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DNA is the chromophore for several biologic consequences of ultraviolet-B (UVB) irradiation including suppression of delayed contact hypersensitivity (Hart *et al*, 1977; Applegate *et al*, 1989; Kripke *et al*, 1992), and cyclobutane pyrimidine dimers, particularly thymine dimers, are the most abundant photoproducts produced in response to this environmental mutagen (Setlow and Carrier, 1966; Cooke and Johnson, 1978; Sutherland *et al*, 1980). Recently, thymidine dinucleotides (pTpT), the substrate for thymine dimer formation, have been shown to simulate several UVB effects (reviewed in Gilchrest and Eller, 1999).

Treatment of cultured melanocytes and melanoma cells with pTpT increases tyrosinase gene expression and melanin content in these cells to a similar degree and with a similar time course as for UVB exposure (Eller *et al*, 1994, 1996; Pedeux *et al*, 1998). Topical application of pTpT to guinea pig skin results in a dose-dependent tanning response with kinetics and histologic changes closely resembling those induced by UVB (Eller *et al*, 1994, 1996; Pedeux *et al*, 1998). In cultured pigment cells, pTpT also increases binding of α-melanocyte stimulating hormone (Eller *et al*, 1996), again closely mimicking the increase following UVB exposure (Bolognia and Pawelek, 1988; Bolognia *et al*, 1989). That pTpT evokes these cellular responses by mimicking DNA damage is suggested by the fact that restriction enzymes and certain radiomimetic chemicals, both known to incise DNA and not known to have other effects on cells, produce the same results (Eller *et al*, 1997). In addition, pTpT induces cell cycle arrest and enhances DNA repair capacity, events mediated at least in part by activation of the tumor suppressor protein and transcription factor p53, also induced by UVB (Healy *et al*, 1994), with subsequent induction of p53-regulated genes such as p21 (Eller *et al*, 1997; Pedeux *et al*, 1998). Deoxyadenine dinucleotides (pdApdA) are far less commonly dimerized by UVB and are far less effective in simulating these UVB effects (Eller *et al*, 1994).

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Because UVB irradiation is a potent inhibitor of the inductive phase of contact hypersensitivity (Jessup *et al*, 1978; Toews *et al*, 1980), and tumor necrosis factor α (TNF-α) is a key mediator of this immunosuppressive effect (Kim *et al*, 1990; Yoshikawa and Streilein, 1990; Rivas and Ullrich, 1994), we sought to determine whether pTpT can inhibit the induction of contact hypersensitivity and upregulate TNF-α expression.

MATERIALS AND METHODS

Mice C57BL/6 (Jackson Labs, Bar Harbor, ME) and transgenic mice (BALB/C background) bearing a chloramphenicol acetyl transferase (CAT)-TNF-α reporter construct (Giroir *et al*, 1992; de Kossodo *et al*, 1995) were used at 4–6 wk of age. Transgenic mice breeders were provided originally by Dr. Bruce Beutler (Howard Hughes Institute, The University of Texas Southwestern Medical Center, Dallas, TX). The CAT reporter transgene (3963 bp) contains the distal coding sequence of lymphotoxin, the enhancer and promoter-bearing sequences lying between the lymphotoxin and TNF-α genes, the TNF-α 5′-UTR, the CAT coding sequence, and the TNF-α 3′-UTR and polyadenylation signal sequences.

Reagents pTpT and pdApdA were purchased from Midland Certified Reagent (Midland, TX), Nutrient Mixture was obtained from Gibco, (Gaithersburg, MD), and lipopolysaccharide (LPS) and actinomycin D were obtained from Sigma (St. Louis, MO). For topical application, pTpT and pdApdA were dissolved in propylene glycol and applied at a concentration of 100 μM per cm^2. For intradermal injection, the dinucleotides were dissolved in sterile saline and injected at a concentration of 2 mM (100 μl per injection). For experiments using cultured dermal fibroblasts, the dinucleotides were added to culture medium at a dose of 200 μM with each medium change. For all experiments, the dinucleotide preparations were coded prior to use, and the code was broken only after measurements were made.

UVB irradiation Light sources were a bank of FS20 fluorescent sunlamps (Westinghouse, Pittsburgh, PA) for studies involving mice, and a solar simulator with a 1 kW xenon arc lamp (Spectral Energy Corporation, Westwood, NJ, and XMN 1000–21, Optical Radiation, Azuza, CA) for studies involving human cells. Sunlamps were placed 46 cm above the target; emission consisted of wavelengths 250–400 nm with a peak at 313 nm and high output primarily in the UVB range (290–320 nm). These lamps deliver an average flux of 1.10 J per m^2 per s UVB as measured by an IL 700 radiometer and SEE 240 UV photodetector with a peak sensitivity of 295 nm (de Kossodo *et al*, 1995). For contact hypersensitivity assays, shaved abdominal skin of C57BL/6 mice was exposed to 200 J per m^2 per d on each of four consecutive days. For experiments using transgenic mice and cultured transgenic dermal fibroblasts, a single dose of 200 J per m^2 was administered. For experiments using SCC12F cells, the solar simulator irradiance was adjusted to 8×10^{-5} W per cm^2 as measured by an IL 1700 A radiometer with a UV probe set at 285 ± 5 nm. Cells were exposed to a single dose of 250 J per m^2 (Friedmann and Gilchrest, 1987; Wintzen *et al*, 1996). Sham-irradiated cultures were handled identically, except for aluminum foil shielding during UV exposure.

Contact hypersensitivity assay C57BL/6 mice were sensitized by topical application of 0.5% dinitrofluorobenzene (DNFB) to shaved abdominal skin (Simon *et al*, 1994). Five days later, dorsal surfaces of left ears were challenged with 0.2% DNFB. Ear thickness was measured prior to challenge and 24 and 48 h after challenge. Incremental ear swelling was computed and the mean peak ear swelling response and standard errors of the mean for each panel of mice were calculated.

SCC12F cell culture The human squamous cell carcinoma line, SCC12F (Rheinwald *et al*, 1983), was provided by Dr. James Rheinwald of Harvard Medical School. These cells were maintained in a 3:1 vol:vol mixture of Dulbecco's modified Eagle's medium and F-12 nutrient mixture (10% fetal bovine serum, 1.8 mM adenine, 10 ng epidermal growth factor per ml). Cultures were kept at 37°C in 7% CO_2 and supplied with fresh medium twice weekly.

ELISA Medium from SCC12F cultures was collected 72 h after pTpT, pdApdA, or diluent supplementation, and centrifuged at 5000 × *g* to eliminate detached cells and debris. The supernatant was transferred to fresh tubes and frozen at –70°C until further analysis. Aliquots of supernatants from duplicate cultures were assayed for TNF-α by ELISA (Biosource International, Camarillo, CA).

Northern blot analysis SCC12F cells were treated for 72 h with 100 μM pTpT or diluent alone. Total cellular RNA was collected with Tri-Reagent (Molecular Research Center, Cincinnati, OH) and isolated according to the manufacturer's instructions. Thirty micrograms of RNA was electrophoresed through a 1% agarose/formaldehyde gel, transferred to a nylon membrane (Hybond N, Amersham, Arlington Heights, IL), and hybridized with ^{32}P-labeled TNF-α or glyceraldehyde-3-phosphate dehydrogenase cDNA (Leverkus *et al*, 1997). Blots were autoradiographed with intensifying screens at –70°C using Kodak X-Omatic AR films. Autoradiograms were scanned with a computerized densitometer (Molecular Dynamics) and quantified using the MD Image Quant 3.2 program.

mRNA stability SCC12F cells were treated for 72 h with 100 μM pTpT or diluent alone. Transcription was then inhibited by adding 5 μg per ml of actinomycin D (time 0) and total cellular RNA was harvested at times 0, 30 min, and 1, 2, 3, 4, and 8 h.

CAT assay CAT activity was measured by thin-layer chromatography (de Kossodo *et al*, 1995). Incubation of the substrate with enzyme was allowed to continue for 4 h, after which CAT activity was quantified using a PhosphorImager. Protein determination was performed using the Bradford reagent (Bio-rad), with reference to a standard of bovine serum albumin.

Statistical analysis Experiments were performed at least three times. The contact hypersensitivity data were analyzed by the two-tailed Student's t test. Data on TNF-α secretion into the medium were analyzed by ANOVA. Mean differences were considered significant for $p < 0.05$.

RESULTS

pTpT inhibits the induction of contact hypersensitivity C57BL/6 mice were subjected to six different treatment protocols (**Fig 1**). Experimental panels consisted of mice treated with pTpT or pdApdA (100 μM in 30 μl of propylene glycol) or vehicle alone on each of five consecutive days through abdominal skin prior to sensitization with DNFB. Positive controls consisted of mice that were not treated prior to sensitization and of mice irradiated with UVB (200 J per m^2 per d on each of four consecutive days) through abdominal skin prior to sensitization with DNFB. Negative controls consisted of mice treated with the vehicle alone (propylene glycol) prior to

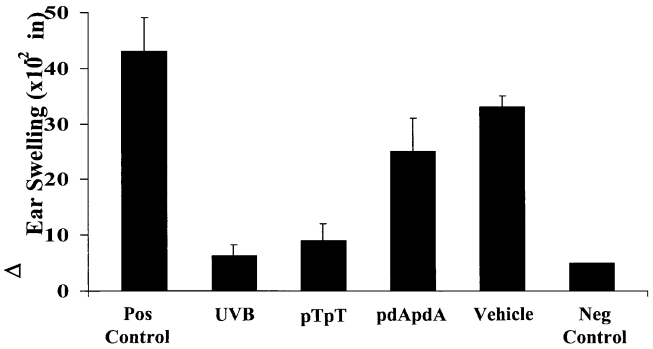


Figure 1. pTpT inhibits the induction of contact hypersensitivity. Panels of C57BL/6 mice (five per panel) were subjected to different treatments prior to sensitization with 0.5% DNFB on day 0. Experimental panels consisted of mice treated topically with pTpT or pdApdA (100 μM daily on days –4, –3, –2, –1, and 0) on abdominal skin prior to sensitization. Positive controls consisted of mice that were not treated prior to sensitization and of mice irradiated with UVB (single dose of 200 J per m^2 daily on days –3, –2, –1, and 0) through abdominal skin prior to sensitization. Negative controls consisted of mice treated with vehicle alone prior to sensitization and of mice that were not sensitized. On day 5, dorsal surfaces of left ears were challenged with 0.2% DNFB. Change in ear thickness was measured and mean peak swelling responses were calculated. DNFB-sensitized mice that were not pretreated or were vehicle-pretreated showed robust ear swelling responses. By contrast, DNFB-sensitized mice pretreated with pTpT or with UVB showed markedly diminished responses ($p < 0.05$, two-tailed Student's t test).

sensitization and of mice that were not sensitized. Ear challenge with hapten 5 d after sensitization revealed robust swelling responses in DNFB-sensitized mice that were not pretreated or were vehicle-pretreated. By contrast, DNFB-sensitized mice pretreated with pTpT or with UVB exhibited markedly diminished ear swelling responses. Mice pretreated with pdApdA showed intermediate responses.

pTpT activates the TNF-α gene Because UVB-induced immunosuppression is known to be mediated largely by TNF-α (Kim *et al*, 1990; Yoshikawa and Streilein, 1990; Rivas and Ullrich, 1994), we examined the effect of UVB *versus* pTpT on the TNF-α gene promoter in an *in vivo* model system (de Kossodo *et al*, 1995). Transgenic mice bearing a CAT reporter linked to the entire TNF-α promoter 5′-untranslated region were first examined in a preliminary time course study, in which CAT activity was 0, 1%, 16%, and 55% acetylation per μg protein $\times 10^{-4}$ at 0, 24, 48, and 72 h, respectively, after irradiation, presumably reflecting a delay between promotor activation following UV exposure and translation of the induced reporter protein. Animals were then subjected to five different treatments and CAT activity was assessed at 72 h (**Fig 2**). LPS, a potent inducer of TNF-α (de Kossodo *et al*, 1995), was used as a positive control. As expected, intradermal injection of LPS (10 μg per ml) induced CAT expression. Consistent with previous studies (de Kossodo *et al*, 1995), a single exposure to 200 J per m^2 UVB induced CAT expression, indicating TNF-α gene promoter activation. Intradermal injection of pTpT (100 μl, 2 mM) also led to activation of the TNF-α gene, whereas injection of pdApdA or vehicle alone did not. Because cultured dermal fibroblasts from these transgenic mice display strong UVB induction of CAT expression (de Kossodo *et al*, 1995), we also compared the effects of 72 h treatment with pTpT or pdApdA with those of UVB irradiation in this assay (**Fig 3**). A single dose of UVB (200 J per m^2) or LPS (10 μM) produced strong CAT expression, as expected. Incubation with pTpT (and to a lesser extent pdApdA) also produced significant TNF-α gene activation, but at lower levels than did UVB or LPS, whereas treatment with vehicle alone failed to activate the TNF-α gene.

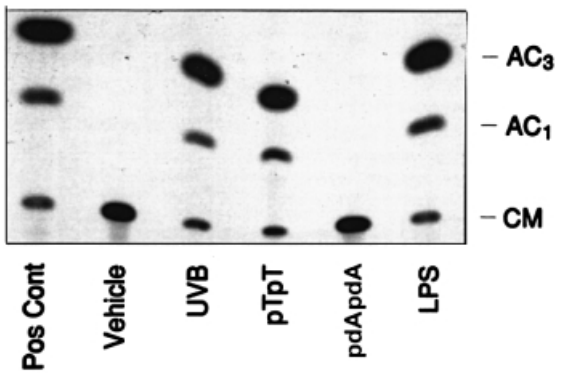


Figure 2. pTpT induces TNF-α promoter CAT activity in skin of transgenic mice. Abdominal skin of transgenic mice bearing a CAT reporter linked to the TNF-α promoter was treated with a single exposure to UVB (200 J per m^2) or with an intradermal injection of pTpT, pdApdA (100 μl, 2 mM), vehicle alone (100 μl), or LPS (10 μg per ml). CAT expression, indicated by the appearance of acetylated substrate, was assayed in freshly harvested skin following UVB exposure or injection of pTpT, LPS, pdApdA, or vehicle alone.

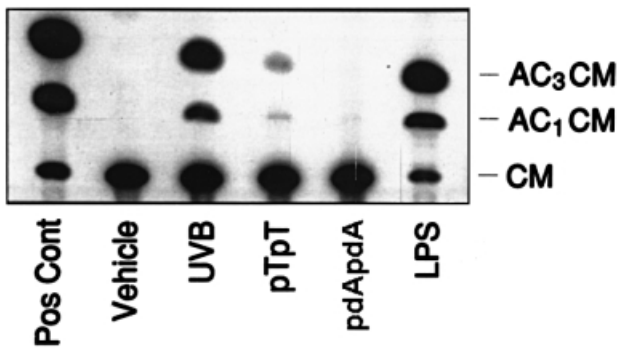


Figure 3. pTpT induces TNF-α promoter CAT activity in dermal fibroblasts from transgenic mice. Dermal fibroblasts from transgenic mice bearing a CAT reporter linked to the TNF-α promoter were cultured. Cultured cells were treated with a single exposure to UVB (200 J per m^2) or incubated with LPS (10 μM), pTpT, or pdApdA (100 μM).

pTpT induces TNF-α protein secretion To examine pTpT effects on TNF-α secretion in a human cell system, we utilized the well- differentiated human squamous carcinoma cell line SCC12F,

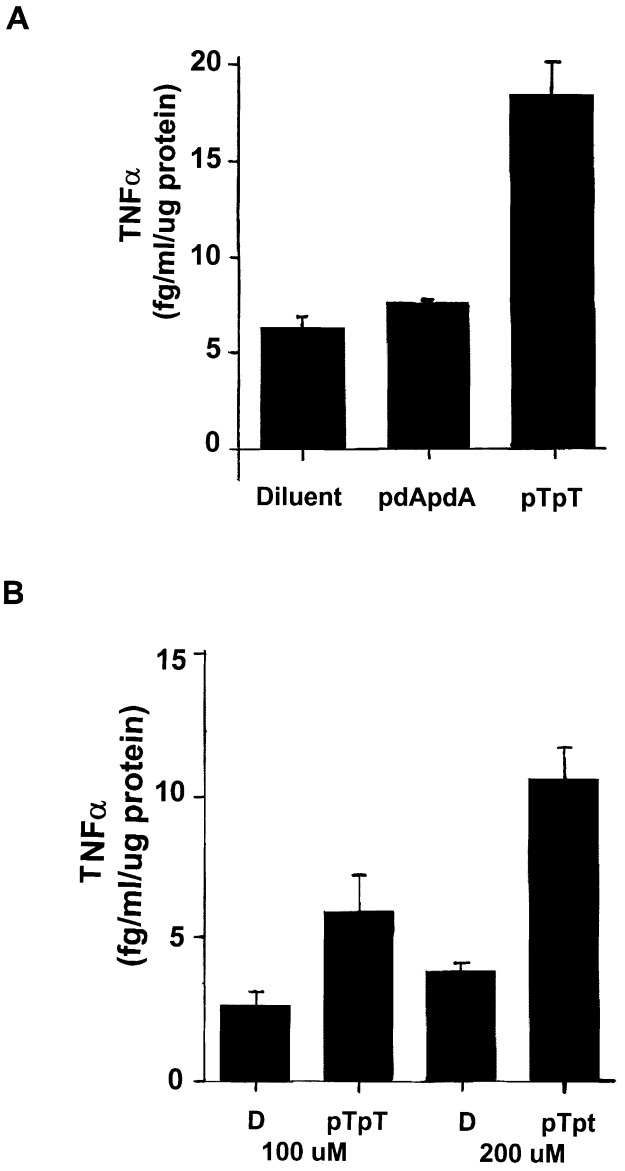


Figure 4. pTpT induces TNF-α protein secretion. (*A*) SCC12F cells were treated for 72 h with pTpT (100 μM), pdApdA (100 μM), or diluent alone; media from duplicate dishes were analyzed by ELISA for TNF-α levels. In five independent experiments, pTpT induced TNF-α secretion 2-fold to 5-fold above baseline levels ($p < 0.002$, ANOVA). One representative experiment is shown. (*B*) SCC12F cells were treated for 72 h with 100 or 200 μM pTpT or an equal volume of diluent alone (D); medium was collected and analyzed by ELISA.

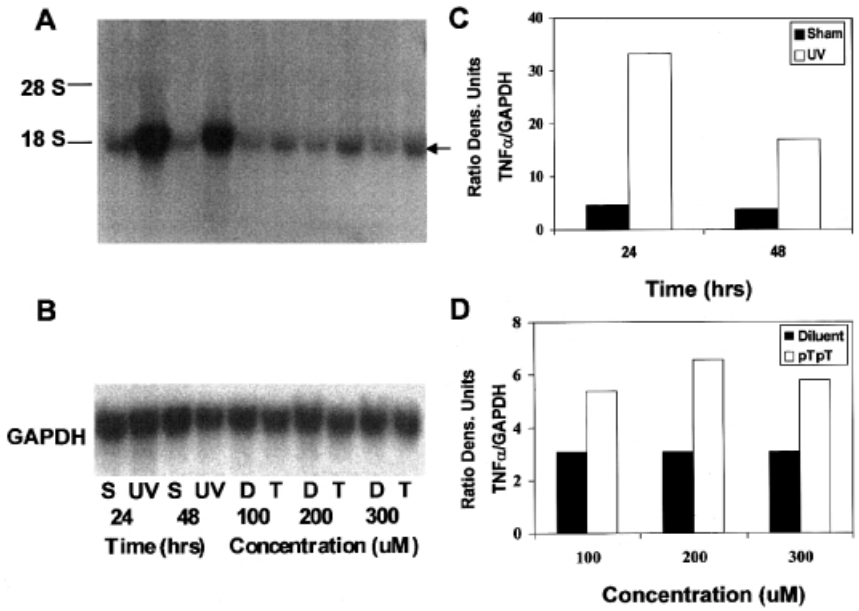


Figure 5. pTpT stimulates TNF-α mRNA expression. Total cellular RNA was collected from SCC12F cells (*A*) 24 and 48 h after treatment with UVB (25 mJ per cm^2) or sham irradiation, or (*B*) 72 h after treatment with pTpT (T) or diluent alone (D). Densitometric analyses (*B* and *C*) depict ratios of the TNF-α signal normalized to the glyceraldehyde-3-phosphate dehydrogenase signal for (*C*) sham and UV-irradiated cells and (*D*) pTpT and diluent.

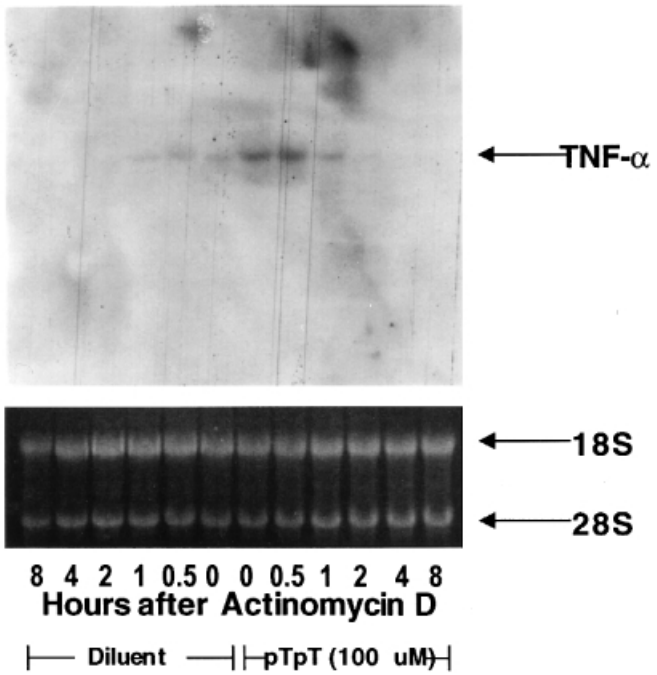


Figure 6. pTpT does not alter stability of TNF-α mRNA. SCC12F cells were treated for 72 h with pTpT or diluent alone; transcription was then inhibited by addition of actinomycin D (time 0). The ethidium-bromide-stained gel shows relative loading of the RNA samples.

known to growth arrest in response to pTpT (Eller *et al*, 1997), and assayed for TNF-α levels in the medium by ELISA. Incubation with pTpT significantly and consistently induced TNF-α protein production 2-fold to 5-fold above the diluent control ($p < 0.02$, ANOVA) (**Fig 4**). Maximal protein secretion was stimulated by a dose of 200 μM pTpT. In contrast, treatment with pdApdA did not significantly increase protein secretion.

pTpT increases TNF-α mRNA level We next determined the effects of pTpT on TNF-α mRNA expression by northern blot analysis. Treatment with pTpT (100–300 μM) was compared with a single dose of UVB irradiation (250 J per m^2) (**Fig 5**). As expected, UVB irradiation upregulated TNF-α mRNA expression. pTpT also upregulated TNF-α mRNA expression, maximally at a dose of 200 μM, but less than UVB. A later upregulation by pTpT was also noted, with increased TNF-α mRNA levels first apparent at 72 h rather than at 24 h, as was observed after UVB irradiation (data not shown).

pTpT does not alter the stability of TNF-α mRNA We have shown previously that physiological doses of UVB induce a more than 20-fold increase in TNF-α mRNA expression in the SCC12F cells (Leverkus *et al*, 1997) and that this augmentation is due primarily to stabilization of mRNA transcripts, which occurs within a few hours after irradiation, followed by modest transcriptional upregulation (Leverkus *et al*, 1998). To determine whether pTpT-inducible TNF-α mRNA upregulation in this squamous cell carcinoma line was due to increased mRNA stability, we measured the half-lives of TNF-α transcripts in SCC12F cells following treatment with pTpT or diluent alone. After 72 h, transcript level was greater in pTpT-treated cells than in controls, as expected. There was a rapid decay of TNF-α mRNA, however, to barely detectable levels as early as 2 h after the addition of actinomycin D in both groups (**Fig 6**) with no significant difference between the calculated half-lives of TNF-α mRNA transcripts in pTpT-treated *versus* diluent-treated cells (35 and 42 min, respectively). Thus, the increased TNF-α mRNA level observed after provision of pTpT is presumptively due to transcriptional upregulation, as in murine fibroblasts (see above), and appears similar in magnitude and in time course to the transcriptional component of UVB-induced upregulation of this transcript in keratinocytes (Leverkus *et al*, 1998).

DISCUSSION

Our studies demonstrate that topical application of pTpT to mouse skin prior to hapten sensitization inhibits the induction of contact hypersensitivity as effectively as UVB irradiation. Because UVB induces TNF-α expression (de Kossodo *et al*, 1995; Leverkus *et al*, 1997, 1998) and TNF-α has been implicated by several laboratories as a key mediator of UVB-induced immunosuppression (Kim *et al*, 1990; Yoshikawa and Streilein, 1990; Rivas and Ullrich, 1994), we further examined the effect of pTpT on expression and secretion of this cytokine. Intradermal injection of pTpT stimulated CAT readout in the skin of mice transgenic for this bacterial enzyme under control of the TNF-α promoter, as did addition of pTpT to cultured fibroblasts of these animals, indicating that pTpT stimulates transcription of the TNF-α gene *in vivo* and *in vitro*. pTpT also upregulated TNF-α protein secretion and mRNA

expression in a well-differentiated human squamous cell carcinoma line, in all likelihood through transcriptional upregulation, given that there was no detectable change in message stability.

Studies in murine and human macrophages have demonstrated that TNF-α gene expression is regulated by both transcriptional (Beutler *et al*, 1985) and post-transcriptional (Hayes *et al*, 1995) mechanisms. Experiments with murine keratinocytes have shown that different stimuli induce TNF-α mRNA via disparate mechanisms. For example, phorbol myristate activates the TNF-α promoter, whereas nickel sulfate increases mRNA stability, and the two agents together act synergistically to increase the amount of TNF-α mRNA (Lisby *et al*, 1985). UV irradiation increases TNF-α mRNA levels in human epithelial cells (Leverkus *et al*, 1998) and dermal fibroblasts (Hayes *et al*, 1995) via both message stabilization and increased transcription rate, with the former mechanism being quantitatively more important (Leverkus *et al*, 1998). Direct transcriptional upregulation of TNF-α was reported in UV-irradiated human cells bearing a CAT transgene under control of the TNF-α promoter (Kibitel *et al*, 1998), an experimental system in which accelerated repair of UV-induced DNA damage was also shown to decrease TNF-α promoter activity. UV irradiation is known to activate p53 (Lu and Lane, 1993; Lu *et al*, 1996), which in turn transcriptionally upregulates many UV response genes. Conversely, TNF-α has been reported to activate p53 (Klefstrom *et al*, 1997; Donato and Perez, 1998; Schafer *et al*, 1998; Yeung and Lau, 1998), possibly creating a positive feedback loop. Activation of p53 has also been shown to be responsible for many of pTpT's effects on gene expression (Eller *et al*, 1997). Because upregulation of TNF-α by pTpT occurs after more than 48 h, however, whereas p53 activation by pTpT is observed in cells within 8 h (Eller *et al*, 1997), the TNF-α increase induced by pTpT is unlikely to be due to a direct transcriptional effect of activated p53. Interestingly, recent work with cells expressing a dominant negative mutant p53 suggests that p53 protein level, irrespective of its transcriptional activity, may modulate TNF-α levels,[2] as for example by sequestering TATA box binding proteins (Seto *et al*, 1992; Mack *et al*, 1993), a mechanism similarly invoked for the delayed p53 upregulation of tyrosinase after pTpT stimulation.[3] UVB irradiation can mediate its biologic effects through direct interaction with nuclear DNA (Freeman *et al*, 1989), through direct activation of transmembrane receptors (Cohen and DeLeo, 1993; Devary *et al*, 1993), and through other extra-nuclear mechanisms (Black *et al*, 1997; Moss *et al*, 1997). Such multiple mechanisms of action are consistent with previous findings that UV upregulates TNF-α expression both by rapidly stabilizing the mRNA transcript and by an increase in gene transcription (Hayes *et al*, 1995; Kibitel *et al*, 1998; Leverkus *et al*, 1998). Our data demonstrate that pTpT augments TNF-α mRNA transcriptionally but only after a delay of at least 48 h, with no detectable change in mRNA half-life. In combination with earlier work suggesting that both UVB and pTpT activate p53 (Lu and Lane, 1993; Lu *et al*, 1996; Eller *et al*, 1997; Maeda *et al*, 1999), a nuclear transcription factor, and that magnitude of TNF-α induction correlates inversely with the rate or extent of DNA repair after UV irradiation (Kibitel *et al*, 1998), these data imply that pTpT mimics those effects of UVB mediated through photon interactions with genomic DNA but not those effects resulting from photon interactions with cell membranes or other extra-nuclear cellular components. This is consistent with previous studies suggesting that DNA is a major target for UVB-induced immunosuppression (Sutherland *et al*, 1980; Applegate *et al*, 1989) and with our working hypothesis that dinucleotides and other small single stranded DNA fragments, known to enter the nucleus, mimic excised UV-induced photoproducts or intermediates in the excision repair process, and activate a cellular response, similar to the SOS response in bacteria, that then evolves over several days (Eller *et al*, 1997; Gilchrest and Eller, 1999; Goukassian *et al*, 1999). The fact that patients with xeroderma pigmentosum, who are deficient in DNA repair, and XPA (–/–) mice have stronger inflammatory and immunosuppressive responses to UVB irradiation than normal controls (Miyauchi-Hashimoto *et al*, 1996) does not contradict this model. For example, despite the lack of recognition or excision of DNA photoproducts, p53 activation in UV-irradiated xeroderma pigmentosum cells is induced by DNA strand breaks generated during replication of a damaged template (Nelson and Kastan, 1994). DNA replication in the presence of unrepaired photolesions in xeroderma pigmentosum cells thus appears to provide single stranded DNA fragments, the presumptive signal for p53 activation, that may in fact be more persistent than excised photoproduct-containing DNA fragments in normal cells, accounting for their sometimes prolonged and exaggerated UV responses.

The comparable ability of pTpT and standard UV doses to inhibit development of contact hypersensitivity *in vivo versus* the greater ability of UVB to induce TNF-α, the presumed principal mediator, in our *in vitro* experiments has three possible explanations. First, the cell types in which pTpT induction of TNF-α was measured may not be as responsive to the dinucleotide as the murine skin cells principally responsible for TNF-α release *in vivo*. In general, for example, fibroblasts appear less sensitive to pTpT than keratinocytes (Eller *et al*, 1997), presumably because of less avid uptake (Eller, Hadshiew, and Gilchrest, unpublished data), whereas keratinocytes are presumably the major source of TNF-α in skin (Kock *et al*, 1990) and rapidly concentrate oligonucleotides in the nucleus (Noonberg *et al*, 1993; White *et al* 1999a, b; Eller, Hadshiew, and Gilchrest, unpublished data). Second, pTpT-induced TNF-α release from SCC12F cells, a squamous cell carcinoma line with overexpressed (Eller *et al*, 1997) and presumptively mutated p53, may be less than from normal human or murine keratinocytes, particularly if the response is p53 dependent. Finally, although unlikely, it is possible that most of the pTpT-induced suppression of contact hypersensitivity is due to release of cytokines other than TNF-α, or to as yet unknown mechanisms, whereas UVB-induced immunosuppression in contrast is principally TNF-α-mediated.

In summary, our findings expand the spectrum of UV effects mimicked by small DNA fragments such as pTpT to include inhibition of the induction of contact hypersensitivity and activation of the TNF-α gene. Moreover, pTpT, the dinucleotide sequence that participates in thymine dimer formation, was far more active than the comparably sized dinucleotide pdApdA, which is similarly almost inactive in pigmentation assays (Eller *et al*, 1994), as is the mononucleotide pT (Pedeux *et al*, 1998). These results support the hypothesis put forward by our group and colleagues (Smith and Fornace, 1997) that DNA photoproducts and/or their repair intermediates trigger many of the biologic consequences of UV radiation and suggest that pTpT may provide ''unless phototherapy'' for immune-mediated UV-responsive skin disorders. Such an alternative therapeutic modality might avoid conventional phototherapy's principal side-effects of photocarcinogenesis and photoaging that result from UV-induced DNA mutations (Hart *et al*, 1977; Brash and Haseltine, 1982; Stern *et al*, 1984, 1997; Brash, 1988; Gilchrest *et al*, 1999).

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