Ultraviolet Irradiation Suppresses T Cell Activation via Blocking TCR-Mediated ERK and NF-κB Signaling Pathways

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UV irradiation is carcinogenic and immunosuppressive. Previous studies indicate that UV-mediated alteration of APCs and induction of suppressor T cells play a critical role in UV-induced immune suppression. In this study, we show that UV irradiation can directly (independently of APCs and suppressor T cells) inhibit T cell activation by blocking TCR-mediated phosphorylation of ERK and IκB via overactivation of the p38 and JNK pathways. These events lead to the down-modulation of c-Jun, c-Fos, Egr-1, and NF-κB transcription factors and thereby inhibit production of cytokines, e.g., IL-2, IL-4, IFN-γ, and TNF-α, upon TCR stimulation. We also show that UV irradiation can suppress preactivated T cells, indicating that UV irradiation does not only impair T cell function in response to T cell activation, but can also have systemic effects that influence ongoing immune responses. Thus, our data provide an additional mechanism by which UV irradiation directly suppresses immune responses. The Journal of Immunology, 2005, 175: 2132–2143.

Ultraviolet Irradiation Suppresses T Cell Activation via Blocking TCR-Mediated ERK and NF-κB Signaling Pathways

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UV irradiation is one of the most prominent natural carcinogens, largely affecting human health, e.g., by inducing skin cancer. UV exposure causes DNA damage that, if not repaired, generates mutations, particularly in the tumor suppressor gene p53 (1, 2). Strong evidence has also shown that exposure to UV irradiation significantly impairs resistance to various infectious agents such as bacteria, parasites, viruses, and fungi (3). Importantly, the immunosuppressive effect of UV irradiation is not restricted to skin-associated infections, but is also apparent in systemic (non-skin-associated) infections (3). Down-modulation of immune responses by UV irradiation has been considered to be a major risk factor for carcinogenesis (4–6).

Although UV light is known to induce skin cancer, UV light has been used as an immunosuppressive therapy in a variety of diseases, including allograft rejection and graft-vs-host disease. The effects of UV radiation on cells of the immune system have been shown to be dependent on both dose and wavelength. UVC (200–280 nm) and UVB (280–315 nm) wavelengths are the most potent, with less biological effects observed for UVA (315–400 nm) irradiation (7). The most commonly used radiation technique is UVB. However, there were also studies showing that UVC is a more effective wavelength or, at least, is as efficient as UVB irradiation tested for suppression of contact hypersensitivity in animal models (8, 9). In addition, a longer survival of human alloskin grafts was observed in a clinical investigation using UVC techniques (10).

The immunosuppressive effect of UV irradiation was first identified in experiments showing that mice exposed to subcarcinogenic doses of UV irradiation failed to reject highly antigenic grafts of UV-induced skin tumors (4, 11). Although this phenomenon has been known and studied for >25 years, the immunologic mechanisms by which tumor tolerance is induced are still not completely understood. Previous studies have shown that UV irradiation may impair the function of APCs (5, 12). APCs play a pivotal role in starting Ag-specific immune reactions. Therefore, a defect in Ag presentation may account for UV-induced immune depression. Another line of studies showed that UV irradiation may induce formation of suppressor T cells (11, 13, 14), and, more recently, that NK T cells from UV-irradiated donor mice could function as suppressor T cells (14, 15).

Of important note is that UV exposure leads to impaired resistance to infections (3). During infections, Th cells play an orchestrating role in the regulation of effective immune responses by secreting various cytokines. Th1 cells produce IFN-γ, IFN-α, and lymphotoxin responsible for cell-mediated immunity. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and play a major role in Ab-dependent immune responses (humoral immunity). Significant suppression of both humoral and cellular immunity after UV exposure has been shown in experimental animal models (16–19). Although this phenomenon has been explained by impairment of APCs and induction of suppressor T cells, a direct role of UV irradiation in regulation of T cell functions has not yet been investigated. Therefore, in this study, we asked whether UV irradiation could directly suppress T cell activation and, thereby, suppress cytokine expression in T cells.

It has previously been shown that UV irradiation of mammalian cells causes activation of several transcription factors, including the early growth response factor Egr-1, the proinflammatory factors c-Jun and NF-κB with subsequent effect on transcription of many genes (20–22). Egr-1, c-Jun, and NF-κB are known to be involved in the activation of several important cytokine genes, including the growth factor IL-2 (23, 24), the Th1 effector cytokines IFN-γ (25, 26) and TNF-α (27, 28), and the Th2 effector cytokine IL-4 (29, 30). One would expect that UV irradiation, which induces activation of Egr-1, c-Jun, and NF-κB, would promote expression of those cytokine genes. In this study, we show that UV irradiation of peripheral blood T cells induces certain levels of Egr-1, c-Jun, and NF-κB. However, UV irradiation alone...
A direct role of UV irradiation in the suppression of the T cell response.
irradiation in two donors (Fig. 1A). Stimulation of T cells via TCR resulted in high levels of cytokine production (Fig. 1A). However, when T cells were pre-exposed to UV, expression levels of the cytokines tested were dramatically down-regulated in a dose-dependent manner. In PMA/ionomycin-stimulated T cells, the production of the cytokines was higher, and consequently, higher doses of UV irradiation (60 J/m²) were required for complete suppression of cytokine expression (Fig. 1A). Similar results were obtained when UV irradiation was applied immediately after T cell stimulation by anti-CD3/anti-CD28 (data not shown).

UV radiation in sunlight may be subdivided into UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm). UVC can be largely absorbed by ozone and oxygen. We, therefore, also examined the effect of UV light on cytokine production with UV 312 nm (UVB). In comparison, an approximate 100 times higher dose of UV 312 nm irradiation was required to achieve a similar reduction of cytokine production than by UV 254 nm (Fig. 1B). Although UV irradiation at high doses may cause death of mammalian cells, at the dose of UV used in our experiments, no significant cell death was seen determined by the MTT assay (Fig. 1C) and by DNA fragmentation (Fig. 1D). Slight inhibition of the cell cycle (G2/M arrest) was observed when the cells were irradiated with UV 312 nm higher than 200 J/m² and UV 254 nm higher than 20 J/m² (Fig. 1D). These experiments indicate that UV irradiation can directly (independently of APCs) suppress T cell activation.

UV suppresses cytokine transcription in T cells

We next analyzed mRNA expression levels of IFN-γ, IL-2, and IL-4 in peripheral blood T cells after exposure to different doses of UV irradiation. Quantitative real-time PCR analysis showed that UV irradiation strongly suppresses cytokine mRNA expression in peripheral blood T cells. Consistent with the protein production data (Fig. 1B), ~100 times stronger inhibition of cytokine mRNA expression by UVC (254 nm) was obtained than by UVB (312 nm) (Fig. 2A). Kinetic analysis of mRNA expression of IFN-γ and IL-4 in peripheral blood T cells showed that UV 254 nm irradiation completely blocked mRNA expression right at the time of the transcription start point. The depressed mRNA levels were slightly recovered 8 h after PMA/ionomycin stimulation in UV 254 nm irradiated T cells (Fig. 2B). In comparison, the inhibitory effect of UV 312 nm was much weaker than the one of UV 254 nm. Approximate by 70% inhibition of IFN-γ and IL-4 mRNA expression by UV 312 nm was observed at the early time point (2 h) of PMA/ionomycin stimulation. However, after 4 h, the depressed

![FIGURE 1](http://www.jimmunol.org/) UV irradiation inhibits cytokine production in peripheral blood human T cells. A, Freshly isolated human peripheral blood T cells were exposed to different doses of UV (254 nm) irradiation, and subsequently stimulated with anti-CD3/anti-CD28 Abs or with PMA/ionomycin, as indicated. After 24-h stimulation, the supernatants were analyzed for cytokine production by ELISA. Non-ind., Indicates noninduction. Data are representative of six separate experiments with T cells from different donors. B, Freshly isolated peripheral blood T cells were treated with either UV 254 nm or UV 312 nm and then stimulated with anti-CD3/anti-CD28. After 24-h stimulation, the supernatants were analyzed for cytokine production by ELISA. Data are representative of two separated experiments. C, Freshly isolated peripheral blood T cells were exposed to different doses of UV 254 nm irradiation. After 24 h, the percentage of surviving cells was determined by MTT assay. The results represent the average of two irradiation assays. D, The cells from B were collected for analysis of apoptotic cell death by FACS for DNA fragmentation.
mRNA levels were completely recovered in UV 312 nm irradiated T cells stimulated with PMA/ionomycin (Fig. 2C). To further investigate the molecular mechanisms of UV-mediated suppression of cytokine production in T cells, we chose the human leukemic T cell line Jurkat as a model system, because Jurkat T cells produce mRNAs of both Th1 and Th2 cytokines and have often been used for studies of various cytokine genes. The kinetics of mRNA expression of IFN-γ, IL-2, and IL-4 in Jurkat T cells was analyzed by RT-PCR. Similar to the results obtained from peripheral blood T cells, UV 254 nm irradiation and then stimulated with PMA/ionomycin for different times. The mRNA expression levels of IFN-γ and IL-4 were analyzed by real-time PCR. C. The IL-4 and IFN-γ mRNA expression levels were analyzed from peripheral blood T cells pre-exposed to a single dose (250 J/m²) of UV 312 nm irradiation.

**FIGURE 2.** UV irradiation inhibits cytokine mRNA expression in activated T cells. A. Freshly isolated peripheral blood T cells from healthy donors were irradiated with different doses of UV 254 nm or UV 312 nm and immediately stimulated with anti-CD3/anti-CD28 Abs. After 4-h stimulation, total RNA was prepared and analyzed for cytokine mRNA expression levels by real-time PCR. Results were internally confirmed by the comparative cycle count (Ct) against β-actin as the standard gene. B. Peripheral blood T cells were pre-exposed to a single dose (30 J/m²) of UV 312 nm irradiation and then stimulated with PMA/ionomycin for different times. The mRNA expression levels of IFN-γ and IL-4 were analyzed by real-time PCR. C. The IL-4 and IFN-γ mRNA expression levels were analyzed from peripheral blood T cells pre-exposed to a single dose (250 J/m²) of UV 312 nm irradiation.

**UV inhibits T cell activation-induced c-Jun, Egr-1, and NF-κB expression**

Egr-1, c-Jun, and NF-κB transcription factors that can be activated by UV irradiation (20–22) are thought in turn to activate cytokine genes such as IFN-γ, TNF, IL-2, and IL-4. However, we did not detect any increase in cytokine production upon UV exposure (Figs. 1 and 2). Therefore, we asked how UV irradiation could, on the one hand, activate transcription factors involved in cytokine gene activation and, in contrast, suppress transcription of these genes. Thus, we first examined expression levels of Egr-1 and c-Jun in UV-irradiated T cells. Freshly isolated peripheral blood T cells were UV irradiated and then stimulated with anti-CD3/anti-CD28 or PMA/ionomycin. Two hours after stimulation, the expression of endogenous c-Jun and Egr-1 proteins was examined by immunoblotting. High levels of expression of c-Jun and Egr-1 were seen in peripheral blood T cells stimulated by either PMA/ionomycin or anti-CD3/anti-CD28. However, expression of these transcription factors was suppressed upon UV irradiation (Fig. 4A).

Therefore, we further investigated the kinetics of UV-mediated suppression of c-Jun and Egr-1 expression. Stimulation of Jurkat T cells led to a rapid expression of c-Jun and Egr-1. UV-induced phosphorylation of c-Jun was also seen at a later time point (120 min after UV exposure) (Fig. 4B). Interestingly, we found that T cell activation-induced expression of Egr-1 and c-Jun was immediately blocked upon UV irradiation. In controls, we showed that UV irradiation did not influence protein levels of tubulin or the constitutively expressed transcription factor SP-1 (Fig. 4B). Therefore, UV irradiation impairs expression of Egr-1 and c-Jun upon T cell activation.

We next examined nuclear expression levels of c-Jun, c-Fos, Egr-1, and the NF-κB subunit p65 in UV-irradiated T cells. Nuclear extracts were prepared from Jurkat T cells pre-exposed to
different doses of UV irradiation and subjected to immunoblotting and EMSA. In agreement with previous studies (20–22), we observed that UV irradiation alone induced a low nuclear level of Egr-1, c-Jun, and NF-κB. However, stronger induction of these transcription factors was seen after T cell activation, and their expression was reduced upon UV irradiation in a dose-dependent manner (Fig. 5A). UV irradiation is known to cause phosphorylation of c-Jun (22, 34), and, indeed, an increase in phosphorylated forms of c-Jun was observed in UV-irradiated T cells (Fig. 5A, indicated by arrows). EMSA showed that UV-mediated reduction of nuclear fractions of Egr-1, c-Jun, and p65 correlated with reduced DNA-binding activity of these factors (Fig. 5B). As controls, expression levels of the constitutive transcription factors Sp1, YY-1, and NF-Y were not affected by UV irradiation.

To further confirm that inhibition of Egr-1, c-Jun, and p65 by UV irradiation would lead to suppression of transcriptional activity of these factors in activated T cells, we performed transfection studies with luciferase reporter constructs containing multiple copies of the consensus DNA-binding elements for Egr-1, AP-1, NF-κB, and the constitutive transcription factor NF-Y. As expected, UV irradiation suppressed the transcriptional activities mediated by these elements in a dose-dependent manner, with the exception of AP-1, whose transcriptional activity was slightly enhanced at the low dose (5 J/m²) of UV irradiation (Fig. 5C). This may be explained by the fact that although UV irradiation reduces expression levels of c-Jun, it increases c-Jun activity via phosphorylation. UV irradiation did not influence the protein levels of NF-Y; however, a slight reduction of NF-Y-mediated transcription was observed (Fig. 5C). Similar results were obtained with a plasmid construct containing 5×SP-1 DNA binding sites (data not shown), indicating that UV irradiation might also interfere with the basal transcriptional machinery. The above data demonstrate that UV irradiation may directly suppress transcriptional activation in TCR-stimulated T cells via suppression of several transcription factors such as Egr-1, AP-1, and NF-κB.

**UV blocks the T cell activation-induced ERK pathway**

In T cells, three major groups of MAPK, the ERK, JNK, and the p38 MAPK, were shown to be involved in the T cell immune response (35). The ERK pathway primarily activates mitogenic signals (36) and has been shown to regulate Egr-1 and c-Jun expression in various cell types (27, 28). The JNK pathway increases transcriptional activity of AP-1 by binding to and phosphorylating c-Jun (34). To test whether UV irradiation influences the MAPK pathway initiated after T cell stimulation, UV-irradiated or unirradiated Jurkat T cells were stimulated for 15–240 min. The MAPK activities were analyzed by immunoblotting with Abs.
against phosphorylated ERK (p-ERK), p38 (p-p38), and JNK (p-JNK). In correlation with the kinetics of Egr-1 and c-Jun expression in UV-treated Jurkat T cells (Fig. 4B), kinetic analysis of the effect of UV irradiation on ERK activation showed that UV irradiation immediately blocked activation-induced phosphorylation of ERK (Fig. 6A, left panel). UV irradiation alone, at least at the doses used in our experiments, did not induce p-ERK expression. In contrast, UV irradiation rapidly induced phosphorylation of p38 and JNK. In comparison, T cell stimulation led to a very weak induction of JNK in Jurkat T cells (Fig. 6A, left panel). Similar to Jurkat T cells, UV irradiation strongly induced phosphorylation of p38 and JNK in peripheral blood T cells (Fig. 6A, right panel). UV irradiation also further enhanced T cell activation-induced phosphorylation of p38 and JNK and suppressed the T cell activation-induced phosphorylation of ERK in peripheral blood T cells. These experiments demonstrate that UV irradiation especially inhibits the ERK pathway in activated T cells.

**UV blocks the T cell activation-induced NF-κB pathway**

In resting T cells, NF-κB remains in an inactive state sequestered by cytoplasmic IκB proteins. Numerous stimuli including cytokines, phorbol esters (e.g., PMA), and T cell activation lead to phosphorylation, ubiquitinylation, and degradation of IκB proteins with subsequent translocation of the DNA-binding subunits of NF-κB into the nucleus (37). Kinetic analysis of the effect of UV irradiation on IκB phosphorylation showed that pre-exposure to UV resulted in an inhibition of IκB phosphorylation upon T cell activation (Fig. 6B). At the doses used, UV irradiation alone did not induce p-IκB. Inhibition of IκB phosphorylation by UV irradiation corresponded with reduction of IκB degradation. Analysis of the IκB phosphorylation state in peripheral blood T cells showed that UV irradiation significantly inhibited IκB phosphorylation (Fig. 6C). Reduced NF-κB-binding activity was also observed in UV irradiated peripheral blood T cells (Fig. 6D). These results suggest that inhibition of IκB phosphorylation may account for UV-induced suppression of NF-κB activation in response to T cell stimulation.

**UV blocks c-Jun, Egr-1, and NF-κB expression in preactivated T cells**

The above-mentioned experiments show that pre-exposure to UV irradiation impairs the T cell response to TCR stimulation. We further investigated whether UV irradiation could suppress expression of Egr-1, c-Fos, and c-Jun in preactivated T cells. Thus, Jurkat

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2 Abbreviation used in this paper: p, phosphorylated.
NF-κB-induced overactivation of p38 and JNK suppresses ERK and NF-κB. Taken together, these data demonstrate that UV irradiation may block phosphorylation of IκBα in prestimulated Jurkat T cells (Fig. 7B). To further determine the functional effect of UV irradiation on preactivated T cells, a Jurkat T cell line bearing an integrated NF-κB-dependent luciferase reporter (3xκB-Luc) was used as a test system for NF-κB activity. The 3xκB-Luc Jurkat T cells were prestimulated with PMA/ionomycin for 30–60 min and then irradiated with 5 or 15 J/m² UV 254 nm. Eight hours later, luciferase activities were determined. T cell stimulation strongly induced NF-κB-dependent luciferase expression. Consistent with the phosphorylation states of IκB, UV irradiation inhibited the T cell stimulation-induced NF-κB-dependent transcription (Fig. 7C). Taken together, these data demonstrate that UV irradiation may suppress an ongoing T cell immune response.

UV-induced overactivation of p38 and JNK suppresses ERK and NF-κB pathway

From the above experiments, we noticed that UV irradiation induces a strong activation of p38 and JNK. It has been reported that p38 activity may inhibit IκBα phosphorylation and thereby limit NF-κB activity (38). To investigate whether overactivation of p38 by UV irradiation interferes with NF-κB activity, PMA/ionomycin-stimulated Jurkat T cells were treated with UV irradiation in the presence or absence of the widely used p38 and JNK kinase inhibitors SB203580 and SP600125. In agreement with the previous report (38), immunoblotting analysis showed that inhibition of the p38 activity largely prevented UV-mediated suppression of IκBα phosphorylation (Fig. 8A). To obtain a clearer picture about the role of p38 and JNK in regulation of the NF-κB activity, we examined the levels of the NF-κB subunit p50 and p65 expression in the nucleus of Jurkat cells after UV irradiation in the presence or absence of the p38 and JNK inhibitors. In correlation with the recovering levels of p-IκBα in the presence of the p38 inhibitor, UV-induced reduction in p65 and p50 in the nucleus was significantly recovered (Fig. 8, B and C). Thus, p38 is involved in inhibition of NF-κB activity.

JNK, p38, and ERK are activated by distinct sets of MAPK kinase (15). We wondered whether overactivation of p38 and JNK pathway would lead to the attenuation of the ERK pathway. To investigate this, experiments were performed with the p38 and JNK inhibitors. As already observed, UV irradiation strongly induces phosphorylation of p38 and JNK. In comparison, stimulation of Jurkat T cells with PMA/ionomycin led to a much weaker activation of p38 and JNK, which could only be seen after a longer time exposure of the blot (Fig. 8D, and data not shown). Higher levels of p38 and JNK activation were associated with lower levels of ERK activity (Fig. 8D). Inhibition of both p38 and JNK activities strongly restored UV-induced reduction of PMA/ionomycin-induced ERK activation (Fig. 8D). Additional experiments using single inhibitor showed that JNK, but not p38, prevents ERK activation (Fig. 8E). The experiments demonstrate that UV-induced overactivation of p38 and JNK pathway may account for the UV-mediated suppression of the ERK and NF-κB pathway.

The ERK pathway has been shown to activate expression of several transcription factors, including Egr-1 and Jun, in different mammalian cells (27, 28, 39–42). To investigate the role of the ERK pathway in the activation of Egr-1, AP-1, and NF-κB in T cells, we stimulated Jurkat T cells with PMA/ionomycin in the presence or absence of the ERK-specific inhibitor PD98059. In agreement with other studies (27, 28, 39–42), immunoblotting analysis showed that inhibition of the ERK activity led to reduced Egr-1 expression in activated T cells (Fig. 8F). The ERK signaling pathway was also shown to be involved in the activation of Fos and Jun in T cells because...
treatment with the ERK inhibitor significantly down-regulated the expression levels of Fos and Jun (Fig. 8F). In contrast, blocking the ERK pathway did not show any inhibition of T cell activation-induced degradation of IκB. The experiment indicates that UV-mediated suppression of the ERK pathway is responsible for deficient Egr-1 and AP-1 activation.

To further investigate the role of p38/JNK in the UV-mediated suppression of cytokine expression in T cells, p38 and JNK inhibitors were used in ELISA analysis. Freshly isolated blood T cells were UV irradiated in the presence or absence of the p38 and JNK inhibitors SB203580 and SP600125, and cytokine secretion was analyzed 24 h after T cell activation. The experiments showed that inhibition of either p38 or JNK partially restored UV-induced reduction of IL-2 and IFN-γ protein production in primary T cells (Fig. 8G). Inhibition of both p38 and JNK activities resulted in an almost complete restoration of IL-2 and IFN-γ production. These experiments demonstrate that UV-mediated overactivation of p38 and JNK is the main cause of UV-mediated suppression of cytokine expression.

UVC and UVB synergistically inhibit T cell activation

UVC can largely be absorbed by ozone and oxygen and also does not penetrate the skin layer as well as the UVB and UVA. The question arose on whether a very low dose of UVC could synergize with the longer UV wavelength to suppress T cell activation. To investigate this question, Jurkat T cells were transiently transfected with the luciferase reporter plasmids containing either the IL-2 or the IFN-γ promoter, and the transfected cells were treated with combinations of UVB and low doses of UVC (1.25–5 J/m²). The experiment revealed that UVC at very low doses could significantly enhance the suppressive effect of UVB on the IL-2 and IFN-γ promoter activity (Fig. 9, A and B). Immunoblotting analysis showed that the T cell activation-induced expression of Egr-1 and c-Jun was synergistically suppressed by UVB plus low doses of UVC (Fig. 9C). The experiments also revealed that low doses of UVC synergize with UVB to enhance T cell activation-induced phosphorylation of p38. In addition, a tendency in synergic blocking of IκB degradation was also observed in cells treated with...

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**FIGURE 6.** UV irradiation blocks T cell activation-induced phosphorylation of ERK and IκB. A, UV-treated (30 J/m², 254 nm) or untreated Jurkat T cells (left panel) and peripheral blood T cells (right panel) were stimulated with PMA/ionomycin for 15–240 min, as indicated. Total cell lysates were immunoblotted with Abs against p-ERK, ERK-1, p-p38, p38, p-JNK, and JNK-1. Similar results were obtained in three separate experiments. B, UV-treated (30 J/m², 254 nm) or untreated Jurkat T cells were stimulated as in A for different times, as indicated. Total cell lysates were immunoblotted with Abs to p-IκBα, IκBα, and tubulin sequentially. Similar data were obtained in three separate experiments. C, Freshly isolated blood T cells were irradiated with different doses of UV 254 nm and then stimulated with plate-bound anti-CD3 and anti-CD28 Abs for 2 h. Total cell lysates were immunoblotted with Ab to p-IκBα and then stripped for immunoblot to anti-IκBα. Data are representative of two separate experiments with two different donors. D, Nuclear proteins of peripheral blood T cells treated with different doses of UV 254 nm were subjected to EMSA analysis with a 32P-labeled NF-κB-binding DNA fragment. NF-Y-binding activity was taken as equal loading control.
combinations of UVB and low doses of UVC. In contrast to UVC, UVB alone does not induce phosphorylation of JNK and c-Jun. Also, UVC and UVB did not show any synergistic effect on the phosphorylation of JNK and c-Jun. These experiments demonstrate that the immunosuppressive effect of UVB could be enhanced by the cotreatment with low doses of UVC.

**Discussion**

The UV-induced suppression of the adaptive immune responses may be responsible for certain infectious diseases, and is also a major risk factor for skin cancer. Previous research indicates that alterations in APC function (5, 12) and induction of suppressor T cells (11, 14, 15) play a critical role in UV-mediated immune suppression. In this study, we provide an alternative mechanism by which UV irradiation directly down-modulates T cell immune responses. We show that UV irradiation suppresses the ERK and IκB signaling pathways initiated by TCR stimulation and thereby down-regulates T cell activation-induced expression of transcription factors Egr-1, c-Jun, c-Fos, and NF-κB, and their target genes (Fig. 10). In particular, IL-2 is an important T cell cytokine driving the proliferation of T lymphoid cells in the periphery (43). In addition, IFN-γ was considered to be a key cytokine involved in tumor rejection by T cells (44). Thus, direct suppression of T cell activation by UV radiation may account for the earliest impairment of T cell immune responses.

Our experiments demonstrate that UV irradiation could block c-Jun, c-Fos, and Egr-1 protein expression, and hence suppress AP-1- and Egr-1-dependent transcription in preactivated T cells. UV irradiation also strongly induces phosphorylation of c-Jun by activation of JNK. Because the activities of c-Jun can be enhanced by phosphorylation, irradiation by a low dose of UV may increase c-Jun-dependent transcription. In contrast, higher doses of UV irradiation led to suppression of c-Jun-mediated transcription due to limited amounts of c-Jun proteins. We also show that UV irradiation can block phosphorylation of IκBα in preactivated T cells and consequently inhibit NF-κB-dependent transcription. These data indicate that UV irradiation does not only impair T cell function in response to T cell activation, but may also have systemic effects that influence ongoing immune responses.

Stimulation of T cells leads to activation of a group of MAPK kinase, which, in turn, activates ERK, p38, and JNK kinases (15). We show that UV irradiation strongly induces activation of the p38 and JNK pathway in T cells. Activation of the mitogen-activated protein signaling pathways by UVC has also been observed in other cell types (45, 46). We show that inhibitor that blocks JNK activation restored UV-mediated suppression of T cell activation-induced ERK activity. Therefore, overactivation of JNK pathway may account for UV-mediated suppression of the ERK pathway (Fig. 10). We also show that overactivation of p38 pathway may lead to suppression of IκBα phosphorylation and nuclear translocation of the
NF-κB subunits p50 and p65. Thus, p38 activity plays a major role in suppression of T cell activation-induced phosphorylation of IκB and degradation of IκB (Fig. 10).

Exposure to UV irradiation has been shown to induce activation of Egr-1, c-Jun, and NF-κB and their target genes (20–22). In agreement with the previous findings, we also found that UV irradiation alone could increase certain levels of Egr-1, c-Jun, and NF-κB expression in nonstimulated T cells. However, we did not detect substantial elevations of IL-2, IFN-γ, TNF-α, and IL-4 in UV-irradiated T cells. With the exception of two donors, a slightly elevated IL-4 production was seen after a higher dose (60 J/m²) of UV 254 nm exposure. However, this was not seen in other donors. In comparison, TCR stimulation induces much higher levels of c-Jun, Egr-1, and NF-κB expression accompanied with high levels of cytokine expression. Apparently, the levels of transcription factors induced by UV irradiation are not high enough to initiate transcription of cytokine genes in T cells, indicating that UV irradiation alone is not able to activate T cells to produce cytokines.

Studies have shown that UV exposure especially inhibits Th1 immune responses, whereas the role of UV irradiation in regulation of Th2 responses is not well documented (16). One study indicates that APCs from internal lymphoid organs of UV-irradiated mice do not present Ag to Th1, but to Th2 cells. However, the exact mechanisms by which the APCs distinguish between Th1 and Th2 are not known (47). It has been speculated that suppression of Th1 responses by UV irradiation might induce a skewing toward Th2 responses, although recent studies in rodents show suppression of the Th2 immune response as well (18, 19, 48). Our experiments demonstrate that exposure of peripheral blood T cells to UV results in an almost equal impairment of Th1 and Th2 cytokine expression at both the protein and the mRNA levels. Therefore, UV exposure leads to a general inhibition of T cell activation.

The most significant wavelength of UV irradiation with respect to skin cancer lies in the range of 200–400 nm. The short wavelength UV at 200–280 nm can largely be absorbed by ozone and oxygen and does not penetrate the earth’s atmosphere as well as the longer wavelength (280–400). Therefore, the contribution of the short wavelength UV irradiation to the development of skin cancers has often been considered to be
less important. However, the continuous reduction of the ozone layer by environmental pollutants (49–51) implies that exposure to short wavelength UV from thinner ozone layers increases the risk for skin cancer (51, 52). In addition, UVC doses at the 159 mJ/cm² to 11.88 J/cm² have been shown to suppress contact hypersensitivity in animal studies (8, 9). Prolongation of survival of human alloskin grafts was observed in a clinical trial using UVC 11 J/cm² (10). UVC was also indicated to be a more effective wavelength than UVB (8). Our data show that UVC (254 nm) is ~100-fold more potent than UVB (312 nm) in suppressing cytokine expression. A single 5 J/m² UV 254 nm exposure resulted in an 80% reduction of cytokine expression in peripheral blood T cells. The doses used in our studies are much lower than the doses used in animal and clinical studies. Thus, although only a small amount of UVC may reach the dermis, the biological effects might be significant.

FIGURE 9. Suppressive effects of UVB can be enhanced by low doses of UVC. A and B, Jurkat T cells were transiently transfected with the pLuc-IL-2 promoter luciferase reporter plasmid. After overnight recovery, the transfected cells were split and exposed to different doses of UV 254 nm and UV 312 irradiation, as indicated. Luciferase activity was determined after 8-h PMA and ionomycin stimulation. C, Jurkat T cells were treated with UV 254 nm and UV 312 irradiation, as in A. The total cell lysates were immunoblotted with the Abs indicated. Data are representative of two separate experiments.

FIGURE 10. Effects of UV irradiation on TCR signaling cascades. TCR signaling leads to the activation of phosphatidylinositol-specific phospholipase C (PLC)-γ, and subsequently results in the hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 3,4,5-triphosphate (IP₃) and diacylglycerol (DAG). IP₃ production leads to an increase of intracellular free Ca²⁺ levels and consequently to the activation of the Ca²⁺-dependent serine/threonine phosphatase calcineurin, which is involved in the dephosphorylation and nuclear translocation of NF-AT. In parallel, DAG activates protein kinase C (PKC) and MAPK. PKC-θ activates IkB kinase, leading to the phosphorylation and degradation of the IkB and the nuclear translocation of NF-κB (p65/p50). The MAPK signaling pathway involves three major groups of MAPK: the ERK, the p38 MAPK, and the JNK. The ERK pathway can be activated by Ras via the Raf group of MAPK kinases. In contrast, the p38 and JNK MAPK are activated by Rho family GTPases, including Rac. Expression of Egr-1, Jun, and Fos is shown to be regulated by the ERK pathway in T cells. The transcriptional activity of Jun can be further enhanced via phosphorylation by JNK. UVB irradiation leads to strong activation of the p38/JNK pathway. In contrast to UVC, UVB activates p38, but not JNK pathway. Overactivation of p38 and JNK pathways results in suppression of TCR-activated ERK and NF-κB pathway, respectively.
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Disclosures
The authors have no financial conflict of interest.

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