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Ultraviolet Radiation Rapidly Induces Tyrosine Phosphorylation and Calcium Signaling in Lymphocytes

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UV radiation is known to induce lymphocyte nonresponsiveness both in vitro and in vivo. We have found that UV radiation rapidly induced tyrosine phosphorylation and calcium signaling in normal human peripheral blood lymphocytes. In the leukemic T cell line Jurkat and the Burkitt's lymphoma cell line Ramos, UV rapidly induced tyrosine phosphorylation in a wavelength-dependent manner, giving strong signals after UVB and UVC, but not UVA, irradiation. Similarly, in Jurkat cells UV-induced calcium signals were dependent on the dose of UVB or UVC irradiation over a range of 150–1200 J/m^2, but only a small signal was observed for UVA at a dose of 1200 J/m^2. The UV-induced calcium signals were blocked by the tyrosine kinase inhibitor herbimycin A, indicating that they were dependent on tyrosine phosphorylation. Phospholipase C (PLC) γ1 was tyrosine phosphorylated in response to UV irradiation but to a lesser extent than observed after CD3 cross-linking. However, PLCγ1-associated proteins demonstrated to bind to the PLCγ1 SH2 domain were tyrosine phosphorylated strongly after UV irradiation. A similar dose response was observed for the inhibition by herbimycin A of UV-induced calcium signals and UV-induced tyrosine phosphorylation of PLCγ1 and associated proteins. We propose that in contrast to CD3/Ti stimulation, UV aberrantly triggers lymphocyte signal transduction pathways by a mechanism that bypasses normal receptor control.

INTRODUCTION

UV radiation has significant biological effects on a variety of cell types, inducing in mammalian cells the expression of specific genes as part of the UV response, which is believed to protect the cells from permanent DNA damage (Herrlich, 1984; Ronai *et al.*, 1990). The effects of UV radiation on cells of the immune system have been characterized extensively. The effects have been found to be dependent on both dose and wavelength. UVC (200–290 nm) and UVB (290–320 nm) wavelengths are the most potent, with little biological effects observed for UVA (320–400 nm) irradiation unless photosensitizing agents are used (Kripke, 1984). UV irradiation of stimulator cells inhibits mixed lymphocyte culture responses (Pamphilon *et al.*, 1991). T lymphocytes are highly sensitive to UV irradiation, showing markedly reduced proliferation and inhibition of responsiveness to mitogens (Deeg *et al.*, 1989). UV irradiation also prevents development of cytotoxic effector cells (Lynch *et al.*, 1981). UVB irradiation of cellular blood components has been reported to prevent both human histocompatibility leukocyte antigen sensitization and transfusion-associated graft versus host disease (Vanprooijen *et al.*, 1992). UV irradiation also has improved graft survival in a variety of transplantation models (Pamphilon *et al.*, 1991). Patients treated with UVB phototherapy for early-stage cutaneous T-cell lymphoma have achieved clinical remission (Ramsay *et al.*, 1992).

It has been reported that UV irradiation results in increases in the concentration of intracellular free calcium ([Ca^{2+}]$_i$) in peripheral blood lymphocytes (PBL) and Jurkat T cells within 2–3 h (Spielberg *et al.*, 1991),

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[1] Abbreviations used: [Ca^{2+}]$_i$, concentration of intracellular free calcium; mAb, monoclonal antibody; PBL, peripheral blood lymphocyte; PLC, phospholipase C; sIg, surface Ig; TCR, T cell receptor.

whereas $[Ca^{2+}]_i$ responses to phytohemagglutinin are impaired (Cereb *et al.*, 1987). Induction of c-*jun* expression also has been observed after UV treatment (Devary *et al.*, 1991). Although UV irradiation is well known to produce DNA damage, these findings on the inhibition of lymphocyte function and $[Ca^{2+}]_i$ responses suggested that UV irradiation might also act directly on key signal transduction pathways of lymphocytes to give rapid responses. Tyrosine phosphorylation is an early response to stimulation via surface Ig (sIg) in B lymphocytes (Gold *et al.*, 1990) and to stimulation via CD3/Ti and accessory molecules in T lymphocytes (June *et al.*, 1990a; Ledbetter *et al.*, 1991). Tyrosine phosphorylation has been shown to be essential for both T-cell (June *et al.*, 1990b; Mustelin *et al.*, 1990) and B-cell (Lane *et al.*, 1991) activation by the use of tyrosine kinase inhibitors. Tyrosine phosphorylation is essential for the rapid $[Ca^{2+}]_i$ signals observed upon stimulation of sIg (Lane *et al.*, 1991) and the T-cell receptor (TCR) (June *et al.*, 1990b). Therefore, we have examined $[Ca^{2+}]_i$ signaling and tyrosine phosphorylation immediately after UV irradiation of lymphocytes. We have found that UV rapidly induces $[Ca^{2+}]_i$ flux and tyrosine phosphorylation in lymphocytes.

MATERIALS AND METHODS

Cells and Reagents

Fresh PBLs from normal donors were isolated by density gradient centrifugation. Affinity-purified rabbit anti-phosphotyrosine antibodies (Kamps and Sefton, 1988; Dailey *et al.*, 1990), anti-human CD3 monoclonal antibodies (mAb) G19-4 and anti-human CD4 mAb G17-2 (Hansen *et al.*, 1984; Haynes, 1986), rabbit antiserum to phospholipase C (PLC)γ1 (Kanner *et al.*, 1992), and PLCγ1–SH2 fusion protein (Gilliland *et al.*, 1992) have been described previously.

UV Irradiation and mAb Treatments of Cells

UV irradiation was performed utilizing a model 1870 Stratalinker UV cross-linker (Stratagene, La Jolla, CA) with 254-, 302-, and 365-nm lamps. An internal photodetector measured the power output of the UV lamps. Cells (5×10^6 per sample) were irradiated in open tissue culture dishes in phosphate-buffered saline (PBS) (for immunoblots and immunoprecipitations) or RPMI 1640 media (for $[Ca^{2+}]_i$ measurements). The sample path length for irradiation was 1 mm to minimize absorbance of UV light by media. Absorbances of media and PBS over a 1-cm path length at 254, 302, and 365 nm were measured utilizing quartz cuvettes with a spectrophotometer (model DU-7; Beckman, Fullerton, CA). For CD3 stimulation, cells were treated with 10 μg/ml G19-4 mAb.

Measurement of Cytoplasmic Calcium Concentration $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ responses were measured using indo-1 (Molecular Probes, Eugene, OR) and a flow cytometer (model 50HH/2150; Ortho, Westwood, MA) as previously described (Rabinovitch *et al.*, 1986). The histograms were analyzed by programs that calculated the mean indo-1 violet/blue fluorescence ratio versus time. There are 100 data points on the X (time) axis on all flow cytometric data.

Immunoprecipitations and Immunoblots

Cells were lysed on ice with NP-40 lysis buffer (50 mM tris (hydroxymethyl) aminomethane, pH 8, 150 mM NaCl, 1% NP-40, 100 μM sodium orthovanadate, 100 μM sodium molybdate, 8 μg/ml aprotinin, 5 μg/ml leupeptin, 500 μM phenylmethylsulfonyl fluoride) and centrifuged at 13 000 × *g* to remove insoluble material. Immunoprecipitation of PLCγ1 was performed as previously described (Kanner *et al.*, 1992). For immunoprecipitations with SH2–Ig fusion proteins, 5 mM dithiothreitol was added to the lysis buffer before precipitation as previously described (Gilliland *et al.*, 1992). Immune complexes were collected on Protein A-Sepharose beads (Repligen, Cambridge, MA), washed four times with NP-40 lysis buffer, once with PBS, and then subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis followed by immunoblotting. Immunoblots with anti-PLCγ1 (Kanner *et al.*, 1992) and anti-phosphotyrosine immunoblotting (Dailey *et al.*, 1990) were performed as previously described. Antibody binding was detected utilizing $[^{125}I]$ protein A followed by autoradiography.

RESULTS

UV Irradiation Induces Rapid $[Ca^{2+}]_i$ Responses in Lymphocytes in a Dose- and Wavelength-dependent Manner

$[Ca^{2+}]_i$ responses in PBL were initially examined utilizing irradiation with UVC, because this wavelength has been reported to be the most effective at inducing lymphocyte responses (Pamphilon *et al.*, 1991). Over the path length of 1 mm used for these experiments, 59% of UVC, 87% of UVB, and 97% of UVA light was transmitted through the RPMI media used in $[Ca^{2+}]_i$ assays, calculated from absorbances over a 1-cm path length. These values were used to correct for attenuation by the culture medium. Virtually all UV light was transmitted through PBS over a 1-mm path length. As shown in Figure 1A, UVC irradiation with 1200 J/m^2 resulted in a significant and prolonged increase in $[Ca^{2+}]_i$ within several minutes of treatment. A twofold higher dose of 2400 J/m^2 accelerated and increased the $[Ca^{2+}]_i$ response. Because T lymphocytes have been reported to be particularly sensitive to UV irradiation (Deeg, 1988), we compared the responses of $CD4^+$ and $CD4^-$ PBL. As shown in Figure 1B, $CD4^+$ PBL gave a very strong $[Ca^{2+}]_i$ signal immediately upon irradiation, with ~97% of the cells responding. By comparison, $CD4^-$ PBL gave a smaller but significant response that was more gradual, with ~65% of the cells responding. Although elevated $[Ca^{2+}]_i$ levels have been observed several hours after UV irradiation (Spielberg *et al.*, 1991), these results clearly demonstrate that UV irradiation also induces very rapid $[Ca^{2+}]_i$ signals and that $CD4^+$ cells give much stronger and more rapid responses than $CD4^-$ PBL. Because $CD4^+$ PBL gave particularly strong UV-induced $[Ca^{2+}]_i$ signals, we further characterized this response in Jurkat cells. As shown in Figure 2A, UVA irradiation gave only a very small $[Ca^{2+}]_i$ signal. In contrast, UVB irradiation gave a strong $[Ca^{2+}]_i$ signal, with a dose-dependent response over a range of 150–1200 J/m^2 of UVB irradiation. UVC irradiation gave an even stronger signal, again with a dose-dependent response. The dependence of the $[Ca^{2+}]_i$ response on wavelength is in accord with previous reports on the biological effects of UV irradiation on lym-

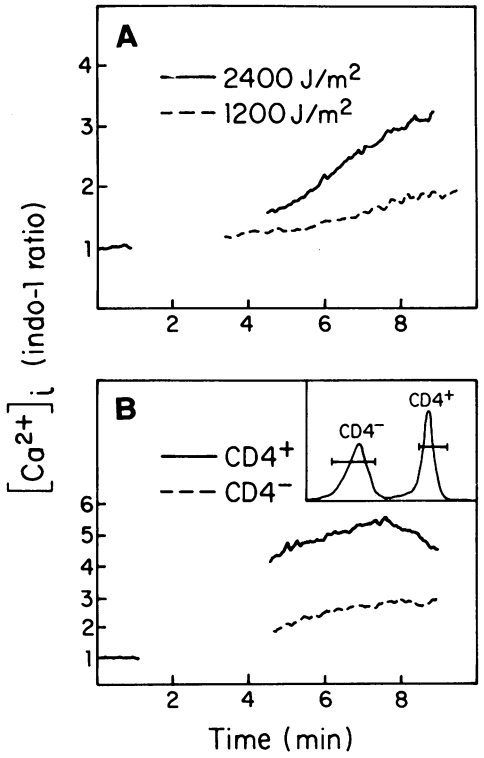

Figure 1. UVC irradiation induces rapid $[Ca^{2+}]_i$ signals in PBL. The baseline level of $[Ca^{2+}]_i$ in PBL was established for 1 min and then cells were irradiated with UVC. Immediately after irradiation, the $[Ca^{2+}]_i$ levels were measured. The blank area in the trace reflects the irradiation period. (A) $[Ca^{2+}]_i$ response of total PBL. (B) $[Ca^{2+}]_i$ response of $CD4^+$ and $CD4^-$ PBL. $CD4^+$ cells were stained with a mixture of G17-2 and G19-2 phycoerythrin conjugates, and the flow cytometer was gated separately on positive and negative cells, as shown in inset. After establishing a baseline, cells were irradiated with 2400 J/m^2 UVC.

phocytes (Pamphilon *et al.*, 1991). UVA irradiation, which has been reported to have little biological effect, gave very slight $[Ca^{2+}]_i$ signals, whereas UVB, which has substantial biological effects, gave strong signals. UVC, which has the greatest biological effects, gave the strongest signal. These rapid and strong $[Ca^{2+}]_i$ signals thus appeared to be well suited as a pathway of signal transduction in lymphocytes to elicit UV effects.

As shown in Figure 3A, the UV-induced $[Ca^{2+}]_i$ also was observed in the presence of ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), demonstrating that Ca^{2+} was released from internal stores as well as entering cells from the outside. The magnitude of the UVC-induced $[Ca^{2+}]_i$ signal was similar to that observed upon stimulation of the TCR with anti-CD3 mAb G19-4 (Figure 3B). The CD3 signal was inhibited when the cells were pretreated with the tyrosine kinase inhibitor herbimycin A, as we have previously reported (June *et al.*, 1990b). The UVC-induced signal was strongly inhibited in the herbimycin A–treated cells (Figure 3B), indicating that like the CD3 signal, the UVC signal was dependent on tyrosine kinase activity. In contrast to the strong $[Ca^{2+}]_i$ signal induced by UVB and UVC, ionizing radiation induced a much smaller signal that nonetheless was reproducibly observed in cells irradiated with 400 cGy (Figure 3C), as well as 200 and 800 cGy in corroborative experiments. This signal was also inhibited by herbimycin A, indicating that the ionizing radiation-induced $[Ca^{2+}]_i$ signal was also dependent on tyrosine kinase activity.

UV Radiation Induces Tyrosine Phosphorylation in Lymphocytes

Because the $[Ca^{2+}]_i$ signals were dependent on tyrosine kinase activity and we had observed previously that ionizing radiation induces tyrosine phosphorylation in B-cell precursors (Uckun *et al.*, 1992), we examined whether UV irradiation would induce tyrosine phosphorylation directly. As shown in Figure 4, UVC irradiation of Ramos cells induced ty-

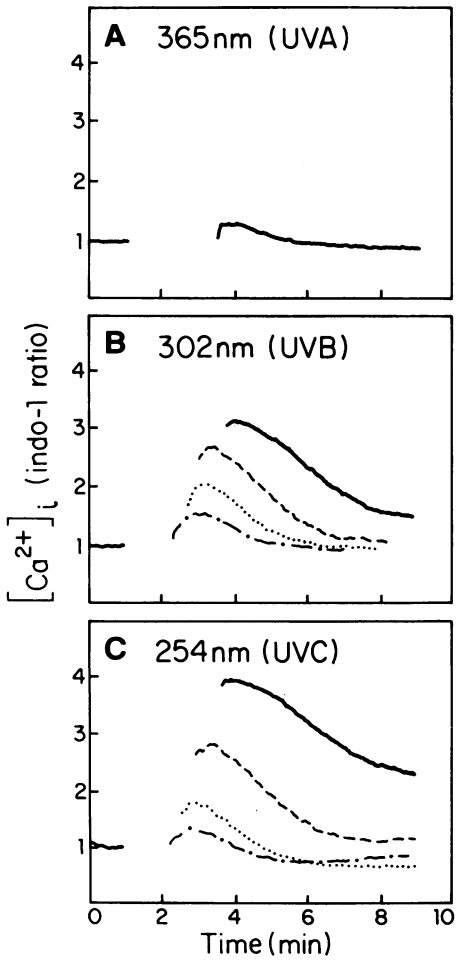

Figure 2. $[Ca^{2+}]_i$ response of Jurkat cells irradiated with (–·–) 150, (···) 300, (---) 600, or (——) 1200 J/m^2 with UVA, UVB, and UVC.

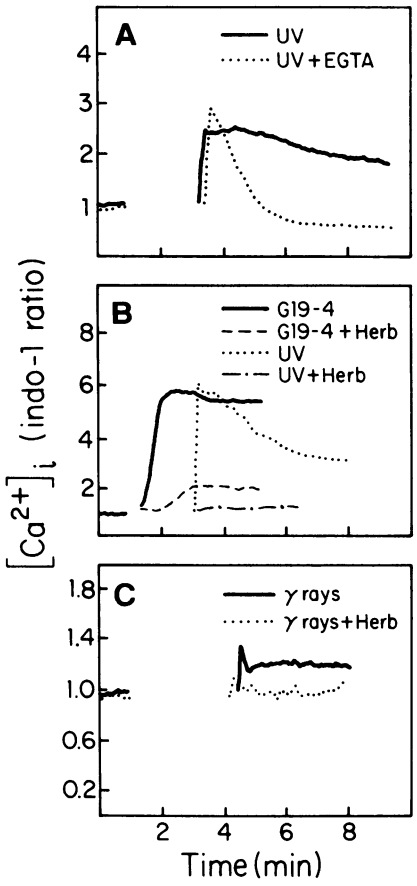

Figure 3. $[Ca^{2+}]_i$ response of Jurkat cells. (A) Cells were irradiated with 1200 J/m^2 UVC in the presence or absence of 5 mM EGTA. (B) Cells were stimulated with G19-4 mAb or 1200 J/m^2 with or without pretreatment with 1 μM herbimycin A (Herb) for 16 h. (C) Cells were irradiated with 400 cGy γ rays with or without pretreatment with herbimycin A as in B.

rosine phosphorylation of 137-, 115-, 94-, 73-, 65-, and 50-kDa cellular proteins in a dose-dependent manner. UVC irradiation also induced tyrosine phosphorylation in Jurkat cells (Figure 4). The pattern of proteins phosphorylated upon UVC radiation was quite similar to that observed upon stimulation via CD3. UVB irradiation also was found to induce tyrosine phosphorylation in Jurkat cells, but UVA irradiation gave little induction (Figure 5). Similarly, in Ramos cells, UVC irradiation induced the highest levels of tyrosine phosphorylation, UVB induced lesser amounts of phosphorylation, and UVA only slightly induced tyrosine phosphorylation (Figure 5). Thus, UV irradiation induces tyrosine phosphorylation in a dose- and wavelength-dependent manner similar to that observed for $[Ca^{2+}]_i$ signaling. In addition to affecting these cell lines, UVC irradiation of human PBL also induced tyrosine phosphorylation (Figure 4).

UV Radiation Induces Tyrosine Phosphorylation of PLCγ1 and PLCγ1 SH2-associated Proteins

Tyrosine kinase–dependent $[Ca^{2+}]_i$ signaling has been found to be mediated by PLCγ1 in T cells (Park *et al.*, 1991; Weiss *et al.*, 1991) and a variety of other cell types (Cantley *et al.*, 1991). A number of tyrosine phosphorylated proteins, including p35/36, associate with PLCγ1 in stimulated cells via the PLCγ1 SH2 domain (Gilliland *et al.*, 1992). We therefore examined tyrosine phosphorylation of PLCγ1 and associated proteins in UV-irradiated Jurkat cells, because Jurkat cells gave strong UV-induced $[Ca^{2+}]_i$ responses and because phosphorylation of PLCγ1 is well characterized in these cells (Weiss *et al.*, 1991). We compared irradiation with 1200 J/m^2 UVC with CD3 stimulation, which had been found to give $[Ca^{2+}]_i$ signals of similar magnitude (Figure 3B). Anti-phosphotyrosine immunoblotting of anti-PLCγ1 immunoprecipitates demonstrated the tyrosine phosphorylation of PLCγ1 and associated proteins upon CD3 stimulation (Figure 6A). Low levels of tyrosine phosphorylation of PLCγ1 could be detected after UV treatment (Figures 6B and 7A), but the amount of tyrosine phosphorylation was less than observed for CD3 stimulation (Figure 6, A and B). Immunoblotting of anti-PLCγ1 immunoprecipitates with anti-PLCγ1 antisera demonstrated that equal amounts of PLCγ1 protein were recovered from UV-irradiated, CD3-stimulated, and untreated cells (Figure 6A). In contrast to the modest level of PLCγ1 tyrosine phosphorylation observed, UV irradiation strongly induced the tyrosine phosphorylation of 125-, 76-, and 35- to 36-kDa proteins that coprecipitated with PLCγ1 (Figure 6, A and B). The precipitation was specific in that these proteins were not precipitated with normal rabbit sera (Figure 6B). Immunoprecipitation with PLCγ1–SH2–Ig fusion protein demonstrated that these tyrosine phosphorylated

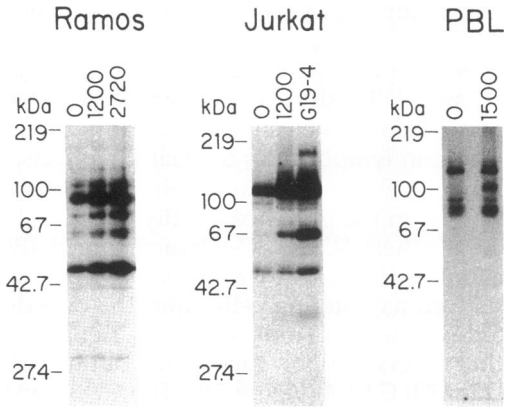

Figure 4. UVC induction of tyrosine phosphorylation. Cells were irradiated with UVC using the doses (J/m^2) indicated or stimulated with G19-4 and then immediately lysed and assayed for tyrosine phosphorylated proteins by immunoblotting as described in MATERIALS AND METHODS.

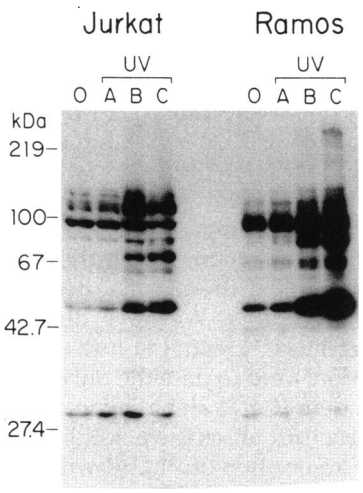

Figure 5. Effect of UV wavelength on induction of tyrosine phosphorylation. Jurkat and Ramos cells were either mock irradiated (0) or irradiated with 2400 J/m^2 UVA, UVB, or UVC and then immediately lysed and assayed for tyrosine phosphorylated proteins by immunoblotting with anti-phosphotyrosine antibodies.

proteins bind to the PLCγ1 SH2 domain and that the overall induction of tyrosine phosphorylation of the 125- and 35- to 36-kDa proteins was similar for both UVC and CD3 stimulation.

UV-induced Calcium Signals and Tyrosine Phosphorylation Show Similar Dose Responses to Herbimycin A

To further examine the link between UV-induced calcium signals and tyrosine phosphorylation, the dose response to herbimycin A was examined in both Ramos B cells and Jurkat T cells. For Jurkat cells, increasing inhibition of tyrosine phosphorylation induced by UV was observed at 0.5–2 μM herbimycin (Figure 7A). In this experiment the UV-induced tyrosine phosphorylation of PLCγ1 was clearly observed in anti-PLCγ1 immunoprecipitations (arrow). The broad band is due to the immunoprecipitating rabbit antibodies. The tyrosine phosphorylation of PLCγ1 and associated proteins was progressively inhibited by increasing doses of herbimycin A over 0.5–2 μM. A similar dose-response was observed for UV-induced $[Ca^{2+}]_i$ signals (Figure 7B). The correlation between the dose responses to herbimycin A for both $[Ca^{2+}]_i$ and tyrosine phosphorylation of PLCγ1 in Jurkat cells supports the hypothesis that UV-induced tyrosine phosphorylation of PLCγ1 and associated proteins leads to the $[Ca^{2+}]_i$ signals. In contrast to the Jurkat T cells, the Ramos B cells showed only a small $[Ca^{2+}]_i$ response to UV that was inhibited moderately by herbimycin (Figure 7C). The dose response of UV-induced whole cell tyrosine phosphorylation to herbimycin A was also different, showing only slight inhibition at 0.5 μM but marked inhibition at 1 μM. These results demonstrate that clear differences exist between the UV responses of these T- and B-cell lines, raising the possibility that different tyrosine kinases may be involved. B cells predominantly express PLCγ2 rather than PLCγ1 (Coggeshall *et al.*, 1992; Hempel *et al.*, 1992), which also could contribute to differences in the $[Ca^{2+}]_i$ responses between the Ramos and Jurkat cells.

DISCUSSION

Two key signal transduction pathways in lymphocyte regulation are tyrosine phosphorylation and calcium signaling. We have shown that UV irradiation directly induces $[Ca^{2+}]_i$ signals and tyrosine phosphorylation in T and B lymphocytes. Furthermore, the UV induction of these signals was found to parallel the wavelength dependence observed for UV immunomodulatory effects. The biologically active UVB and UVC wavelengths were found to induce strong $[Ca^{2+}]_i$ signals and tyrosine phosphorylation, whereas UVA irradiation induced only weak signals, even when high doses of UVA irradiation were used. In addition, UV signaling responses were observed to occur in a dose-dependent manner. Our findings indicate that UV-induced $[Ca^{2+}]_i$ signaling and tyrosine phosphorylation are well suited to be major mechanisms in addition to DNA damage by which UV irradiation could affect lymphocyte function.

The inhibition of the UV-induced $[Ca^{2+}]_i$ signal by the tyrosine kinase inhibitor herbimycin A indicated that

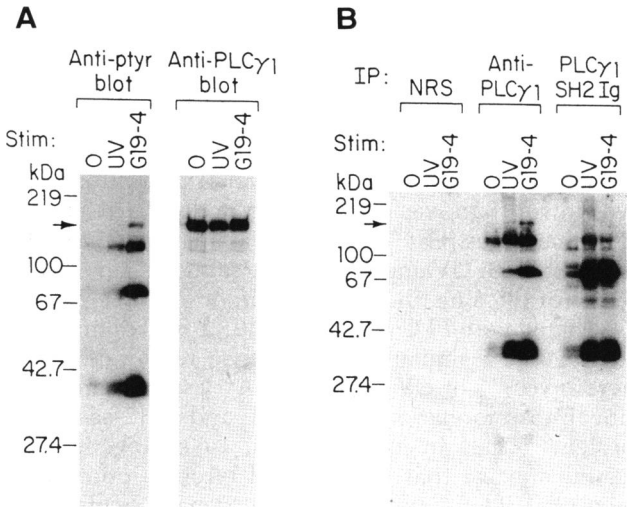

Figure 6. Tyrosine phosphorylation of PLCγ1 and associated proteins. (A) Anti-PLCγ1 immunoprecipitation from Jurkat cells after treatment with G19-4, 1200 J/m^2 UVC (UV), or no treatment (0). Arrow indicates PLCγ1 band on anti-phosphotyrosine and anti-PLCγ1 immunoblots. (B) Anti-phosphotyrosine immunoblot after SDS gel electrophoresis of immunoprecipitates from Jurkat cells utilizing normal rabbit sera (NRS), anti-PLCγ1, and PLCγ1 SH2-Ig.

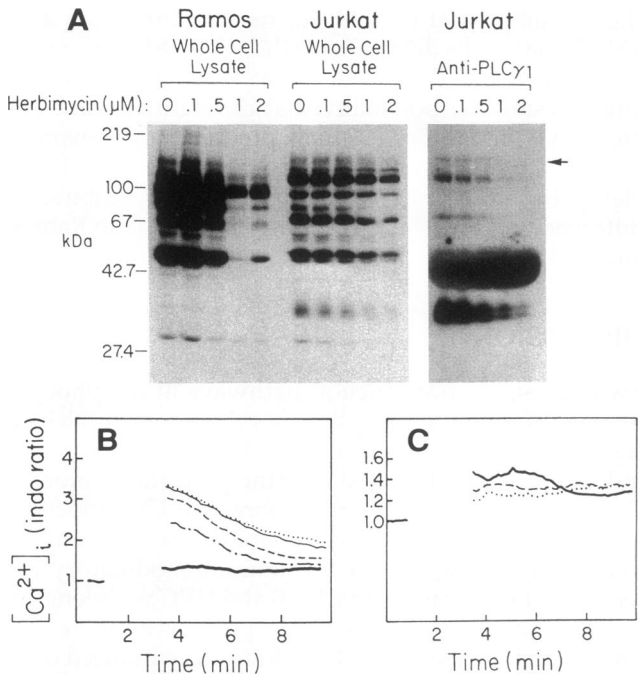

Figure 7. Dose response of herbimycin A inhibition of UV-induced tyrosine phosphorylation and calcium signals. Ramos and Jurkat cells were treated 16 h with the indicated concentrations of herbimycin and then irradiated with 1200 J/m^2 UVC. (A) Antiphosphotyrosine immunoblot of cellular proteins in whole cell lysates and anti-PLCγ1 immunoprecipitates (arrow indicates PLCγ1). (B) UVC-induced $[Ca^{2+}]_i$ response in Jurkat cells pretreated with (——) 0, (· · ·) 0.1, (– – –) 0.5, (–·–) 1, or (——) 2 μM herbimycin A. (C) UVC-induced $[Ca^{2+}]_i$ response in Ramos cells pretreated with (——) 0, (– – –) 1, or (· · ·) 2 μM herbimycin A.

tyrosine kinase activity was essential for the $[Ca^{2+}]_i$ signal. Tyrosine kinases are known to be able to generate $[Ca^{2+}]_i$ signals via the tyrosine phosphorylation of PLCγ1 (Rhee and Choi, 1992). We have shown that UV irradiation can induce tyrosine phosphorylation of PLCγ1 in Jurkat cells, but the level of phosphorylation was lower than was observed upon anti-CD3 stimulation that gives $[Ca^{2+}]_i$ signals of similar magnitude. In contrast, both UV and anti-CD3 treatments gave similar levels of tyrosine phosphorylation of multiple proteins associated with PLCγ1 via the PLCγ1 SH2 domain. We found that a similar dose response for inhibition by herbimycin A of UV-induced $[CA^{2+}]_i$ signals and tyrosine phosphorylation of PLCγ1 and SH2-associated proteins occurs in Jurkat T cells. Taken together, our results suggest that UV-induced tyrosine phosphorylation of PLCγ1 and associated proteins leads to the $[Ca^{2+}]_i$ signal. There is increasing evidence that tyrosine phosphorylation of PLCγ1 alone does not account for the induction of PLCγ1 activity (Benhamou and Siraganian, 1992). Activation of *kit* kinase results in tyrosine phosphorylation of PLCγ1, but this does not lead to a significant increase in the production of inositol phosphate (Lev *et al.*, 1991). Studies with C-terminal deletion mutant epidermal growth factor (EGF) receptors incapable of binding to PLCγ1 via SH2 interactions have demonstrated that association of PLCγ1 with EGF receptor is required for PLC activation in vivo, whereas tyrosine phosphorylation of PLCγ1 without association is insufficient (Vega *et al.*, 1992). Thus, it appears that for productive PLCγ function in vivo, PLCγ must associate via SH2 interactions with other tyrosine phosphorylated proteins at the plasma membrane, such as growth factor receptors. We have demonstrated that the UV-induced $[Ca^{2+}]_i$ signal in Jurkat cells is tyrosine kinase dependent, and to date the only known tyrosine kinase–dependent $[Ca^{2+}]_i$ signaling in Jurkat cells is via PLCγ. Our data thus raises the possibility that in T lymphocytes, the association of PLCγ1 with tyrosine phosphorylated proteins via SH2 interactions may be sufficient to induce PLC activity even when PLCγ1 itself is tyrosine phosphorylated only at low levels. Further analysis, including the identification of the associated proteins, will be required to determine the role of these proteins in PLCγ function in lymphocytes.

While this study was under review, it was reported that UV radiation induces the c-*jun* gene in HeLa cells via a pathway involving activation of src family tyrosine kinases followed by activation of Ha-Ras and Raf-1 (Devary *et al.*, 1992). Thus, UV affects tyrosine phosphorylation signal pathways in cells other than lymphocytes. However, questions of wavelength dependence, the extent of cellular tyrosine phosphorylation, identification of substrates, and induction of calcium signals addressed in the present study on lymphocytes have not been reported for the HeLa cell system. It has been reported that UV-induced activation of pp60$^{c\text{-}src}$ in HeLa cells can be blocked by *N*-acetyl cysteine (Devary *et al.*, 1992), a free radical scavenger. Although we have found previously that ionizing radiation, a free radical generator, induces tyrosine phosphorylation in B-cell precursors (Uckun *et al.*, 1992), UV radiation lacks sufficient energy to ionize water (Halliwell and Gutteridge, 1989). Furthermore, the much stronger induction of $[Ca^{2+}]_i$ signals by UV relative to ionizing radiation indicates a nonidentical mechanism of action. Thus, UV-sensitive chromophores in the cell must transduce the signal. One possibility is that UV-induced DNA damage triggers the phosphotyrosine signal. Comparison of action spectra has led to the hypothesis that UV-induced DNA damage is an intermediate step in the UV induction of expression of human immunodeficiency virus type 1 (HIV-1) and other UV-inducible genes (Stein *et al.*, 1989b). The regulation of p34^{cdc2} tyrosine phosphorylation in response to the state of DNA replication (Smythe and Newport, 1992) provides a precedent for linking the state of cellular DNA to tyrosine kinase signal transduction. However, the rapidity of the tyrosine phosphorylation response that we have observed suggests a role for more direct interaction between UV-

responsive chromophores and tyrosine kinases. One possibility is the generation of free radicals by UV peroxidation of lipids (Black, 1987; Devary *et al.*, 1992). Because src-family kinases are involved in receptor-mediated activation of lymphocytes (Bolen *et al.*, 1992), the determination of the extent to which these and other tyrosine kinases are activated by UV treatment of lymphocytes relative to the HeLa cell system will be important in the understanding of this phenomenon.

Taken together with the recent report on UV induction of src kinases in HeLa cells leading to c-*jun* induction (Devary *et al.*, 1992), the UV induction of calcium signals and tyrosine phosphorylation in lymphocytes offers a new potential mechanism in addition to DNA damage for UV effects on cells. Although UV-induced signal transduction is most likely to elicit rapid cell responses to UV radiation, it also could be of potential importance in longer term responses as well. For example, it has been found that when calcium signals are induced under nonmitogenic conditions in T lymphocytes by modulation of CD3 or pulsing with calcium ionophores, the cells enter a state of nonresponsiveness (Davis *et al.*, 1989; Jenkins *et al.*, 1987). The induction of calcium signals in T cells by UV irradiation potentially could have similar effects. Thus, it will be of value to determine the relative importance of UV-induced signal transduction and UV-induced DNA damage in the inhibition of normal lymphocyte function. Our results and those for HeLa cells (Devary *et al.*, 1992) indicate that it also will be of interest to determine the extent to which UV can induce tyrosine phosphorylation or calcium signaling in nonlymphoid cells. In addition to UV induction of c-*jun* expression in HeLa cells, leading to transcription of genes containing AP-1 binding sites in their promoters (Devary *et al.*, 1991), UV irradiation also activates NF-κB and induces expression of HIV-1 (Stein *et al.*, 1989a,b). We recently have found that tyrosine kinase activity is required for activation of NF-κB activity by ionizing radiation (Uckun *et al.*, 1993). Ionizing radiation is known to induce c-*jun* expression (Sherman *et al.*, 1990) by a mechanism dependent on tyrosine kinase activity (Chae *et al.*, 1993). These findings suggest that UV-induced tyrosine phosphorylation could potentially be required for UV activation of NF-κB and HIV-1, as well as the induction of c-*jun* expression. It is therefore of interest to determine whether UV-induced phosphotyrosine or calcium signals lead to expression of UV-inducible genes in a variety of cell types.

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