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Antiapoptotic role of p38 mitogen activated protein kinase in Jurkat T cells and normal human T lymphocytes treated with 8-methoxypsoralen and ultraviolet-A radiation

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A combination of 8-methoxypsoralen and ultraviolet-A radiation (320–400 nm) (PUVA) is used for the treatment of T cell-mediated disorders, including chronic graft-versus-host disease, autoimmune disorders, and cutaneous T-cell lymphomas. The mechanisms of action of this therapy, referred to as extracorporeal phototherapy, have not been fully elucidated. PUVA is known to induce apoptosis in T lymphocytes collected by apheresis, however no information is available concerning the underlying signaling pathways which are activated by PUVA. In this study, we found that PUVA treatment of Jurkat cells and human T lymphocytes up-regulates the p38 MAPK pathway but not the p42/44 MAPK or the SAPK/JNK signaling networks. The use of a pharmacological inhibitor selective for the p38 MAPK pathway, SB203580, allowed us to demonstrate that this network exerts an antiapoptotic effect in PUVA-treated Jurkat cells and T lymphocytes from healthy donors. Moreover, the effect of SB203580 was not due to a down-regulation of the Akt survival pathway which was not activated in response to PUVA. These results may suggest that p38 MAPK-dependent signaling is very important for the regulation of survival genes after exposure to PUVA. Since the therapeutic effect of PUVA seems to depend, at least in part, on apoptosis, further studies on the apoptosis signaling networks activated by this treatment might lead to the use of signal transduction modulators in combination with PUVA, to increase the efficacy of this form of therapy.

Keywords: apoptosis; Jurkat; p38 MAPK; PUVA; survival.

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Introduction

Extracorporeal photopheresis (ECP) is a novel immunomodulatory treatment by which leukocytes, collected by apheresis, are exposed to 8-methoxypsoralen (8-MOP) and ultraviolet (UV) -A radiation, then reinfused into the patients. The combination of 8-MOP and UVA irradiation, commonly referred to as PUVA, is used to treat diseases such a cutaneous T cell lymphoma[1] and other T cell-mediated immune disorders, including pemphigus vulgaris, scleroderma, systemic lupus erythematosus, and chronic graft-versus host disease (GvHD).[2,3] Moreover, it has been employed to reverse allograft rejection after organ transplantation.[4]

8-MOP is a naturally occurring tricyclic aromatic compound with a planar structure that helps it intercalate between nucleic acid base pairs. Upon exposure to UV-A radiation, 8-MOP, which is photoactive, intercalates into double-stranded nucleic acids, resulting in the formation of photoadducts with pyrimidines in cellular DNA.[5] The psoralen monoadducts formed in DNA can further react photochemically with a pyrimidine base on the complementary strand of DNA, thus leading to crosslinks that are believed to be the primary cause of PUVA-induced cell killing.[6]

Although the mechanisms of action of PUVA treatment await full elucidation, evidence suggests it induces apoptosis in lymphocytes[7,8] or in Jurkat T lymphoblastoid cells.[6] Scarce information is available concerning the mechanisms underlying apoptotic cell death effected by PUVA. It has been shown that PUVA treatment of Jurkat T lymphoblastoid cells causes mitochondrial disfunction resulting in the opening of the permeability transition pore and a decrease in $\Delta\psi_m$.[6] We have very recently shown

that PUVA treatment of Jurkat T cells and normal T lymphocytes leads to the activation of both apical caspases − 8 and − 9.[9]

Since the therapeutic effect of PUVA treatment might somehow be related also to the induction of apoptosis,[10] it may be desirable to further our understanding about the signaling cascades which are activated in response to PUVA.

Cellular commitment to apoptosis, or the ability to evade apoptosis in response to genotoxic stress, involves the integration of a complex network of survival and death pathways. Among the best characterized pathways regulating cell survival and cell death are those mediated by the mitogen-activated protein kinase (MAPK) family.[11,12] These enzymes are regulated by a characteristic phosphorelay system in which a series of three protein kinases phosphorylate and activate one another. Several distinct subgroups within the MAPK family have been described. These include: (1) extracellular signal-regulated kinases (ERKs, also referred to as p42/44 MAPKs), (2) c-jun N-terminal or stress-activated protein kinases (SAPK/JNK), and (3) the p38 group of MAPK. While the activities of major MAPK subgroups are subject to modulation upon exposure of different cell types to diverse types of apoptosis inducers, the response seems to be context-dependent, and can differ depending on the system and conditions. Despite these complexities, some important trends have surfaced. The ERK pathway plays a key role in cell proliferation, survival, and differentiation.[13] The SAPK/JNK and p38 MAPK are activated in response to chemical and environmental stress. The SAPK/JNK has largely, but by no means exclusively, been implicated in proapoptotic signaling,[14,15] while p38 MAPK has been linked with either cell death or survival.[16]

With the above in mind, we decided to investigate whether or not PUVA treatment of Jurkat T cells and normal T lymphocytes resulted in the activation of members of the MAPK family. We have found that this form of therapy selectively activated p38 MAPK. p38 MAPK inhibition with SB203580 caused a marked increase in the number of cells which underwent apoptosis in response to PUVA treatment. This finding indicates a possible way to modulate the response to this type of therapeutic treatment.

Materials and methods

Chemicals and antibodies

Bovine serum albumin (BSA), normal goat serum (NGS), mouse monoclonal antibody to β-tubulin, and peroxidase-conjugated anti-mouse or anti-rabbit IgG were from Sigma, St. Louis, MO, USA. The COMPLETE Protease Inhibitor Cocktail, and the Lumi-Light^{Plus} enhanced chemiluminescence (ECL) detection kit were from Roche Applied Science, Milan, Italy. The Protein Assay kit (detergent compatible) was from Bio-Rad, Hercules, CA, USA. The p38 MAPK inhibitor SB203580, the MEK kinase inhibitor PD98059, the phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002, and okadaic acid were purchased from Calbiochem, La Jolla, CA, USA.

The following antibodies were employed in this study, all of them from Cell Signaling Technology, Beverly, MA, USA: rabbit polyclonals to total Akt (#9272), Ser 473 phosphorylated Akt (p-Akt) (#9271), Thr 308 p-Akt (#9275), total p42/44 MAPK (# 9102), Thr 202/Tyr 204 p-p42/44 MAPK (#9101), total p38 MAPK (# 9212), Thr 180/Tyr 182 p-p38 MAPK (# 9211), total SAPK/JNK (# 9252), Thr 183/Tyr 185 p-SAPK/JNK (# 9251), and mouse monoclonal to Thr 180/Tyr 182 p-p38 MAPK (Alexa Fluor 488 Conjugate, # 4551).

Cell culture

Human Jurkat T lymphoblastoid cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (complete medium) in 5% CO_2-95% air at 37°C, at an optimal cell density of 3 to 8×10^5 cells/ml.

Isolation and liquid culture of normal T lymphocytes

Fifty milliliters of peripheral blood from healthy donors were collected in heparin tubes after informed consent, according to institutional guidelines. T cells were isolated by negative selection. Briefly, peripheral blood lymphocytes from healthy donors were obtained by Ficoll-Hypaque gradient separation. Non T cells were labeled with a cocktail of biotinylated monoclonal antibodies, followed by anti-biotin magnetic microbeads (Pan T cell Isolation Kit II, Miltenyi Biotec, Bergish Gladbach, Germany). Non T cells were subsequently eliminated with MACS columns and Vario MACS equipment (Miltenyi). Purification (>95%) was checked by flow cytometry utilizing a fluorescein isothyocianate-conjugated CD3 monoclonal antibody (Beckman Coulter, Miami, FL, USA). Two milliliters of the cell suspension were seeded in Petri dishes. Untreated and PUVA-treated T cells were resuspended at a concentration of 5×10^6/ml cell in RPMI 1640 medium containing 10% fetal bovine serum and phytohemagglutinin (PHA) (0.5 μg/ml, from Sigma).

PUVA treatment

Cells seeded in Petri dishes (10^6/ml) were incubated with 8-MOP (200 ng/ml, Gerotz Pharmazeutica, Vienna,

Austria) in serum free medium for 5 min at room temperature and then exposed to 2.0 J/cm^2 of UV-A light. UV-A irradiation was performed by the PUVA Combi Light irradiator (DERMAT BVBA, Heverlee, Belgium). The apparatus was provided with two UV-A dosimeters and with an air ventilation system, to ensure both an accurate emission of the requested UV-A dose and a constant irradiation chamber temperature (below 25°C). After treatment cells were incubated in complete medium at 37°C for the indicated times.

Flow cytometric analysis

For sub-G1 (apoptotic cells) peak analysis, Jurkat cells and T lymphocytes were harvested by centrifugation at 200 × g for 10 min, fixed with 70% cold (4°C) ethanol for 1h and subsequently stained with propidium iodide (PI, DNA-Prep kit, Beckman Coulter). The subdiploid DNA content was analyzed and calculated using an Epics XL flow cytometer with the appropriate software (Beckman Coulter). At least 10,000 events/sample were acquired.

For detection of p-p38 MAPK, cells (5×10^5/sample), washed in phosphate buffered saline, pH 7.4 (PBS), were fixed in 2% paraformaldehyde for 10 min at 37°C. After pelletting, fixed cells were permeabilized by slowly adding ice-cold 90% methanol and incubating on ice for 30 min. After two washes in PBS, 0.5% BSA (incubation buffer), samples in 90 μl incubation buffer were kept for 30–60 min in the dark at room temperature with 10 μl of mouse monoclonal to Thr 180/Tyr 182 p-p38 MAPK (Alexa Fluor 488 Conjugate). After two washes in incubation buffer, samples were analyzed by flow cytometry with argon laser at 488-nm excitation and 527-nm emission wave lengths (Cytomics FC500 flow cytometer, Beckman Coulter).

Measurement of $\Delta\psi_m$

This was measured using flow cytometry of cells stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1, from Molecular Probes, Eugene, OR). Cells (2×10^6/ml) were incubated for 15 min at 37°C in culture medium with 4 μM JC-1. After washing twice with PBS, cells were immediately analyzed using flow cytometry with argon laser at 488-nm excitation and 527-nm emission wave lengths (Cytomics FC500 flow cytometer, Beckman Coulter).

Protein assay

This was performed according to the instruction of the manufacturer using the detergent compatible Bio-Rad Protein Assay.

Preparation of cell homogenates and western blot analysis

Cells were washed twice in PBS containing the COMPLETE Protease Inhibitor Cocktail + phosphatase inhibitors (1 mM Na_3VO_4, 2.5 mM Na pyrophosphate, 1 mM 2-glycerolphosphate, 25 mM NaF). Cells were then lysed at $\sim 10^7$/ml in boiling electrophoresis sample buffer containing the protease and phosphatase inhibitor cocktails. Lysates were briefly sonicated to shear DNA and reduce viscosity, and boiled for 5 min to solubilize protein. Protein separated on SDS-polyacrylamide gels (SDS-PAGE) was transferred to nitrocellulose sheets using a semi-dry blotting apparatus. Sheets were saturated in PBS containing 5% NGS and 4% BSA for 60 min at 37°C (blocking buffer), then incubated overnight at 4°C in blocking buffer containing the primary antibody. After four washes in PBS containing 0.1% Tween-20, they were incubated for 30 min at room temperature with the appropriate peroxidase-conjugated secondary antibody, diluted 1:5,000 in PBS-Tween-20, and washed as above. Bands were visualized by the ECL method.

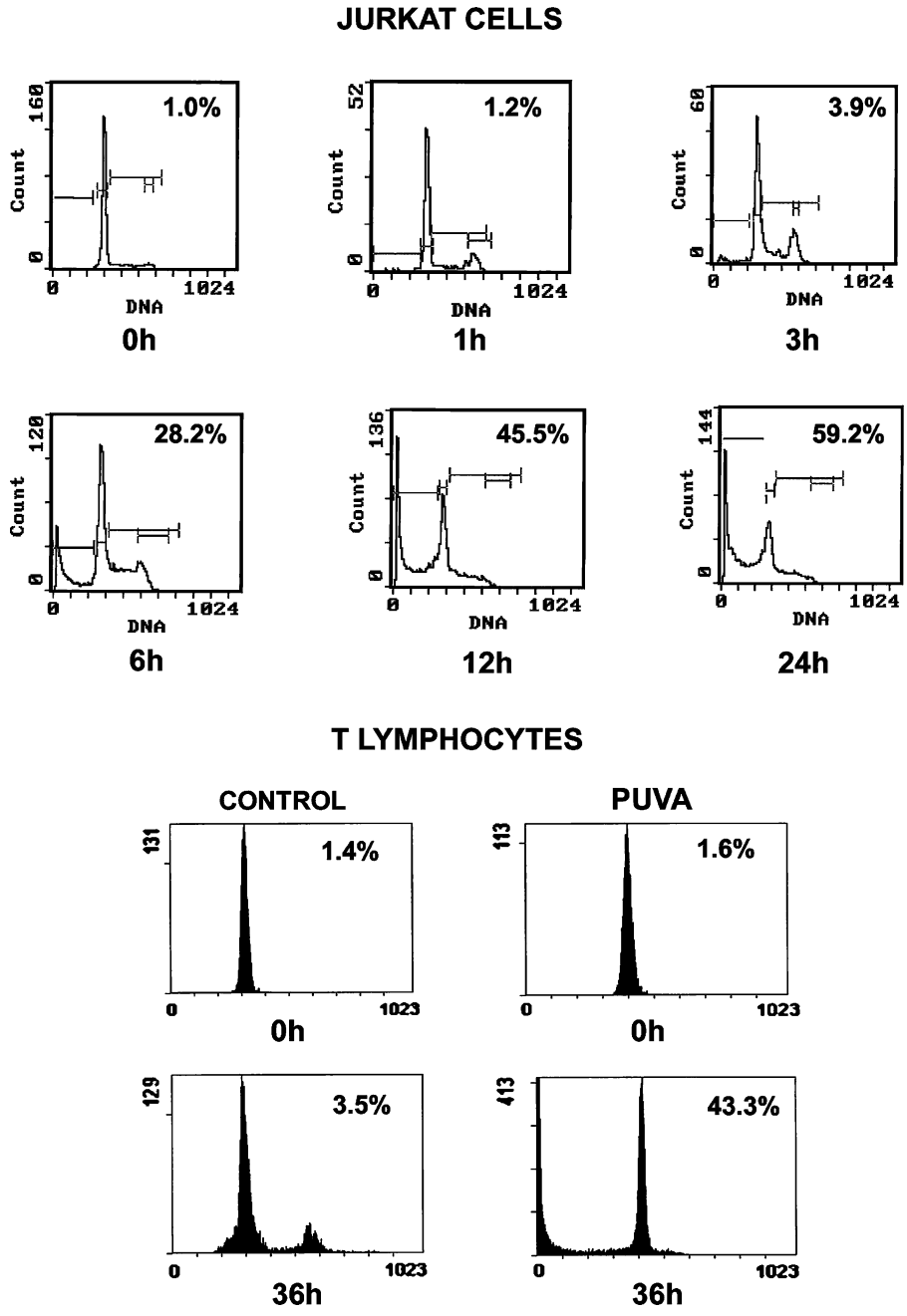
Results

PUVA-induced apoptosis of Jurkat T cells and normal T lymphocytes

In agreement with our own previous results,[9] flow cytometric analysis of PI-stained samples showed that, 6 h after PUVA treatment, there was a significant increase in the number of apoptotic Jurkat cells, evaluated as a sub-diploid peak (Figure 1). The number of apoptotic cells progressively increased at 12 and 24 h from PUVA exposure. We then analyzed for apoptosis PUVA-treated human T lymphocytes from healthy donors. As shown in Figure 1, a significant increase in the number of apoptotic cells began to appear 24 h after treatment. Subsequently, the percentage of apoptotic cells dramatically rose at 36 h. It should be noted that in PUVA-treated Jurkat cells and T lymphocytes we did not detect cells in the G2/M phase of the cell cycle.

As a control, we evaluated whether treatment with either 8-MOP or UV-A alone would induce apoptotic cell death. However, as shown in Figure 2A, no significant increase in the amount of apoptotic cells was measured in Jurkat cells or T lymphocytes when the two treatments were used separately. Moreover, we analyzed by flow cytometry the $\Delta\psi_m$ of Jurkat cells and normal T lymphocytes subjected to PUVA. As presented in Figure 2B, a marked decrease in $\Delta\psi_m$ was detected only if cells had been subjected to the combined treatment. Either 8-MOP or UV-A alone were ineffective.

Figure 1. Flow cytometric evaluation of apoptotic cell death induced by PUVA in Jurkat cells and human T lymphocytes. Samples were subjected to PUVA treatment, incubated for the indicated period of times, fixed, stained with PI, then analyzed by flow cytometry. Representative histograms of three different experiments are shown. The percentage of cells present in the sub-G1 populations (apoptotic cells) is indicated for each istogram.

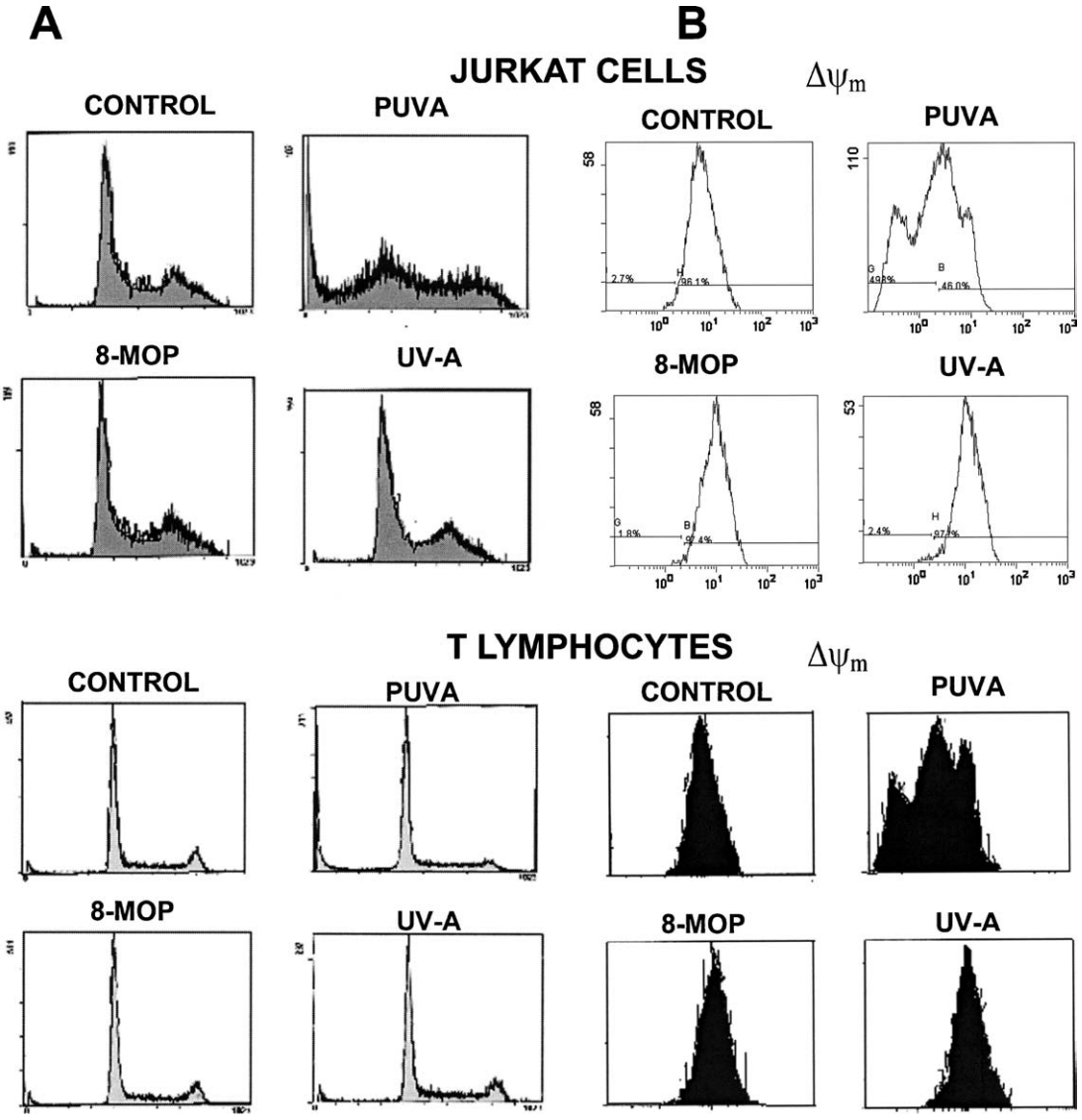


Analysis of MAPK family activation

To test whether PUVA treatment activates MAPKs in Jurkat cells, we examined the phosphorylation of p42/44 MAPK, p38 MAPK, and SAPK/JNK. Western blot analysis with antibodies specific for Thr 202/Tyr 204 p-p42/44 MAPK showed no activation in response to PUVA treatment up to 1 h (Figure 3A). In contrast, we detected a rapid activation of p38 MAPK. Indeed, there was an increase in the tyrosine/threonine phosphorylation levels of this kinase starting 15 min after PUVA exposure. Phosphorylation progressively increased up to 1 h. These phosphorylation levels were well maintained up to 6 h since treatment.

On the other hand, the levels of phosphorylation of SAPK/JNK were unaffected (Figure 3A). Since p38

Figure 2. Flow cytometric detection of apoptosis (A) and of $\Delta\psi_m$ changes (B) in Jurkat cells or normal T lymphocytes, treated with either 8-MOP or UV-A alone. A: samples were subjected to treatments, fixed, stained with PI, then analyzed by flow cytometry. Jurkat cells: samples were analyzed 12 h after treatment. T lymphocytes: samples were analyzed 36 h after treatment. B: for $\Delta\psi_m$ analysis, cells were stained with JC-1 and subjected to flow cytometry as described under Materials and Methods. Analysis was performed 6 h after PUVA treatment of Jurkat cells or after 10 h in case of T lymphocytes. In A and B representative histograms of three different experiments are shown.

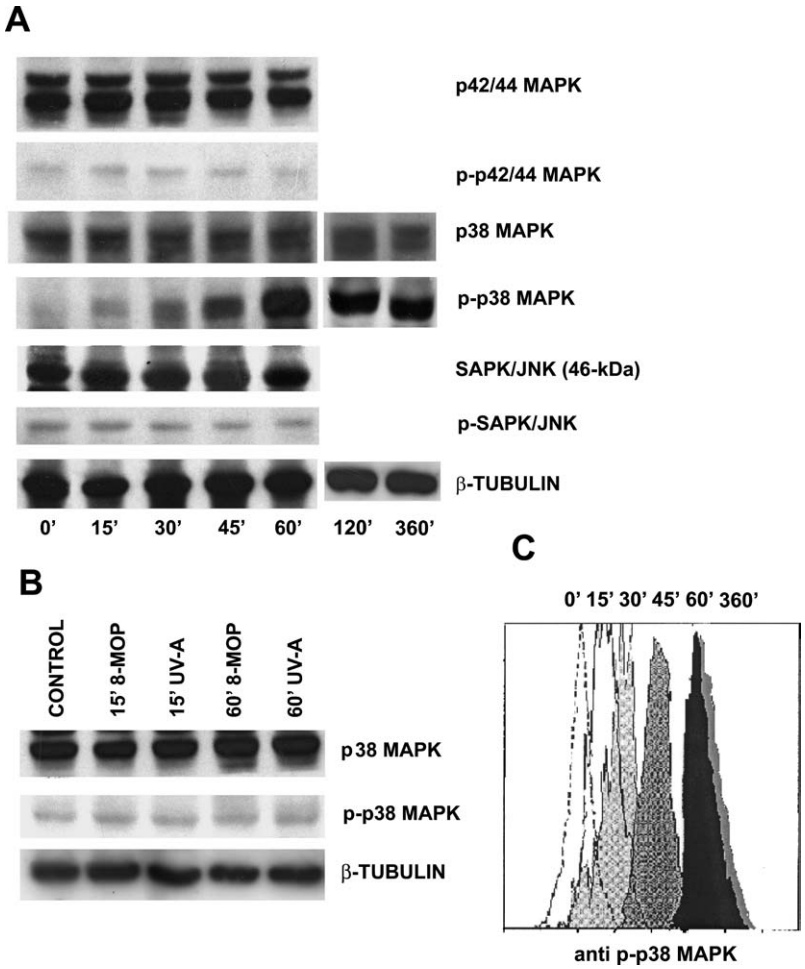


MAPK activation could be due to the effect of UV-A alone[17] and not to the combined action of 8-MOP plus UV-A, we tested if the two treatments, employed separately, resulted in an increase of p38MAPK phosphorylation. However, as shown in Figure 3B, the two treatments, when applied alone, failed to activate p38MAPK in Jurkat cells. Activation of p38MAPK was also investigated by flow cytometry, using an Alexa Fluor 488-conjugated antibody which reacts with p38MAPK only when dually phosphorylated on Thr 180/Tyr182. As shown in Figure 3C, this technique confirmed rapid activation of p38MAPK in response to PUVA treatment of Jurkat cells.

A selective p38MAPK inhibitor enhances apoptosis rate in PUVA-treated Jurkat T cells

To assess the relevance of p38MAPK activation in PUVA-treated Jurkat cells, we employed the pharmacological inhibitor SB203580. Samples were incubated with the inhibitor for 45 min prior to PUVA treatment, then analyzed for apoptosis at various times by flow cytometry.

Figure 3. PUVA treatment activates p38 MAPK in Jurkat cells. A: western blot analysis for p42/44 MAPK, p38MAPK, and SAPK/JNK (total and phosphorylated) performed on cell extracts. Cells were exposed to PUVA, then incubated for increasing periods of time, lysed, and protein was separated by SDS-PAGE. B: western blot analysis for p38MAPK (total and phosphorylated) performed on cell extracts prepared from cells exposed to either 8-MOP or UV-A alone for the indicated times. In A and B 80 μg of protein was blotted to each lane. Immunoreactive bands were visualized by the ECL technique. Immunostaining with a monoclonal antibody to β-tubulin confirmed equal loading. Blots are representative of three separate experiments. C: flow cytometric analysis of p38 MAPK activation. Cells were fixed, permabilized, and stained with an Alexa Fluor 488-conjugated antibody which recognizes p-p38 MAPK. Samples were analyzed by flow cytometry with argon laser at 488-nm excitation and 527-nm emission wave lengths. Representative histograms of three different experiments are shown.



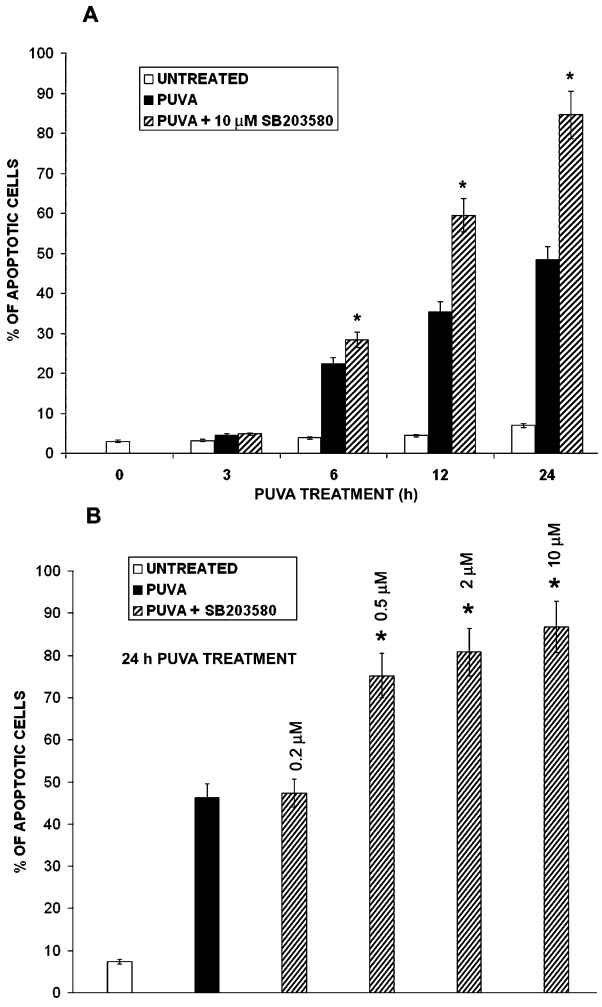
As shown in Figure 4A, already 6 h after treatment the inhibitor (10 μM) was capable of inducing a significant increase in the number of apoptotic cells. A marked increase was also detected at 12 and 24 h after treatment, when the inhibitor raised the percentage of apoptotic cells to almost 85% with respect to the total cell count in comparison to 48% without the inhibitor. PD98059, a pharmacological inhibitor selective for p42/44 MAPK, which was not activated by PUVA, was without effect (data not shown, but see ref.[9])

A SB203580 concentration study performed 24 h after PUVA treatment revealed that, while a concentration of 0.2 μM did not increase the percentage of apoptotic cells, a 0.5 μM concentration induced a significant increase in the number of apoptotic cells in PUVA-exposed samples (Figure 4B). The apoptosis rate further slightly increased when SB203580 concentration was raised to 2 or 10 μM.

SB203580 does not inhibit Akt phosphorylation in Jurkat cells

Since it has been reported that SB203580 may inhibit Akt phosphorylation by blocking phosphoinositide-dependent kinase 1,[18] we sought to clarify this issue in our system. This seems particularly important because Jurkat cells, which lack PTEN expression, have high levels of phosphorylated (active) Akt that may substantially contribute to their survival capability[19,20] and also because our own previous data showed that wortmannin, an

Figure 4. A p38 MAPK inhibitor increases apoptosis in PUVA-treated Jurkat cells. A: SB203580 had been present since 45 min prior to PUVA exposure. Cells were incubated up to 24 h after treatment with PUVA. B: Jurkat cells, treated with increasing concentrations of SB203580, were incubated for 24 h after PUVA exposure. PI-stained samples were then analyzed by flow cytometry for the detection of apoptotic (sub-G1) cells. Results are the mean of three different experiments ± sd. The asterisk indicates significant differences ($p < 0.01$) with respect to samples not treated with SB203580.



inhibitor of the PI3K/Akt pathway, increased the number of apoptotic cells after PUVA treatment.[9] As presented in Figure 5A, a time course analysis of Ser 473 p-Akt and Thr 308 p-Akt levels by western blot did not evidentiate an increased Akt phosphorylation after PUVA treatment up to 60 min. Moreover, PUVA-treated Jurkat cells were incubated up to 16 h in the presence or in the absence of SB203580 and the Akt phosphorylation levels were evaluated by western blot using phosphospecific antibodies (Figure 5B). However, also in this case no changes in Akt phosphorylation were seen. In contrast, incubation of the cells for the same time in the presence of a selective PI3K inhibitor, LY294002 (25 μM), resulted in a dramatic decrease in the phosphorylation levels of Thr 308 p-Akt (Figure 5C).

Furthermore, SB203580 (concentration range 0.2–10 μM) did not affect the cell cycle and apoptosis rate of untreated Jurkat cells, thus demonstrating that its effects on survival were strictly associated with PUVA treatment (Figure 5D).

PUVA treatment activates p38 MAPK also in human T lymphocytes

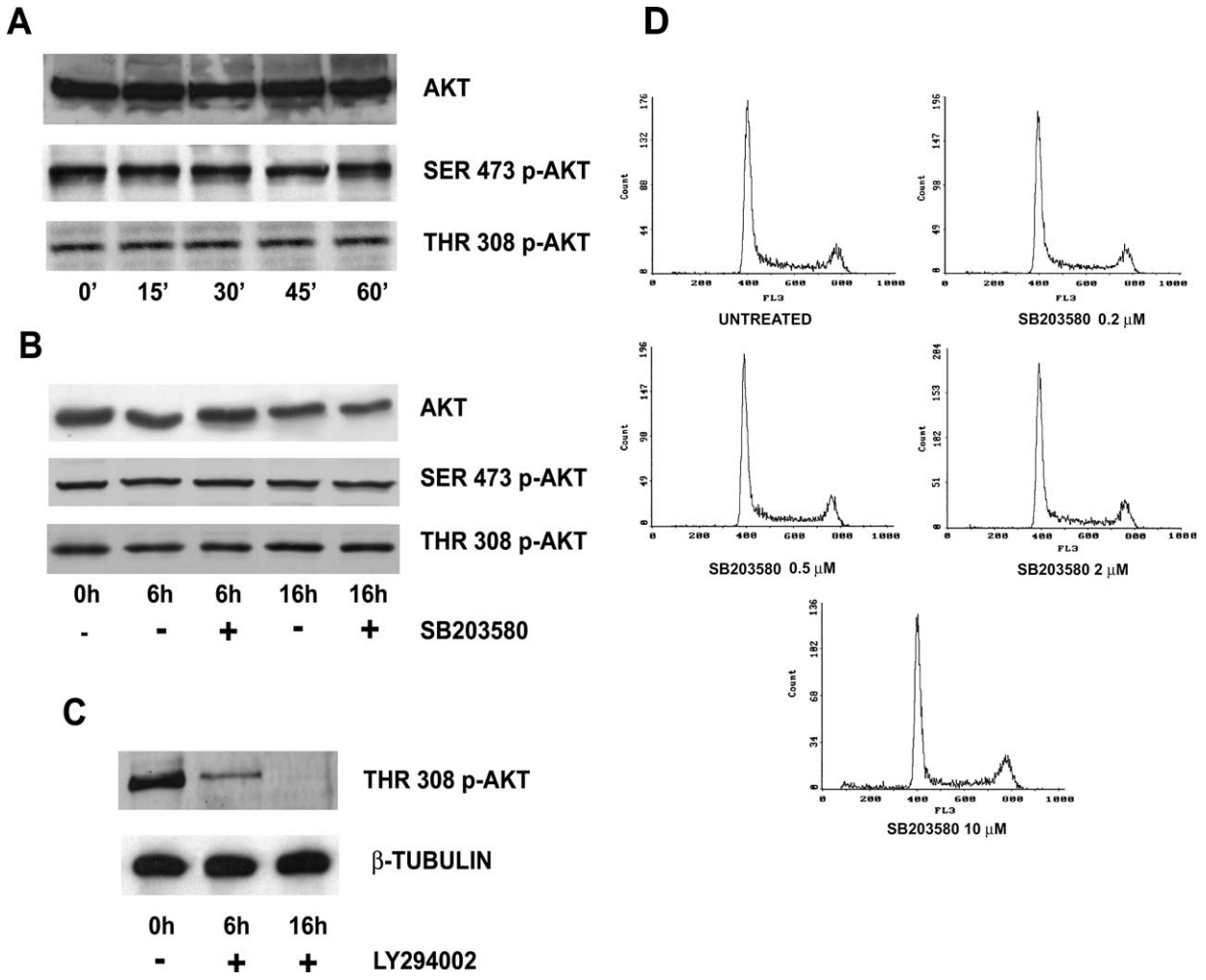
Finally, we investigated whether activation of p38 MAPK was also detectable in PUVA-treated T lymphocytes from healthy donors. As shown in Figure 6A, western blot analysis with phosphospecific antibodies to p-p38 MAPK revealed an increase in the phosphorylation levels starting at 8 h after PUVA treatment. p-p38 MAPK levels increased up to 24 h. Nevertheless, we also detected an increase in the p-p38 MAPK amount in T lymphocytes treated with PHA alone, even if this increase was always less evident that in samples treated with PUVA + PHA. These data were corroborated by flow cytometric analysis at 12h and 24 h with Alexa Fluor 488-conjugated anti-p-p38 antibody (Figure 6B) which showed a higher activation of p38 MAPK in samples exposed to PUVA in comparison to those treated with PHA alone. Finally, the use of SB203580 (0.5–1.0 μM) resulted in a significant increase in apoptotic T lymphocytes in response to PUVA treatment after a 24 h incubation (Figure 6C).

Discussion

ECP treatment has been shown to be of clinical benefit in the treatment of several T cell mediated diseases. The effectiveness of ECP has been explained, in part, by the induction of apoptosis in the treated T lymphocytes (reviewed in[21]). For example, in has been reported that in patients treated with ECP therapy for refractory GvHD, there was T lymphocytes apoptosis associated with increase in circulating interleukin-10 and interleukin-1 receptor antagonists suggesting that ECP modifies autologous lymphocytes by inducing a process of apoptosis that activates monocytes and macrophages.[22] Hence, the processing and presentation of apoptotic T cells antigens from clones of pathogenetic T cells by macrophages and dendritic cells could explain the induction of anticlonotipic activity by ECP.[23] Indeed, there is evidence that ECP could induce the generation of clone-specific suppressor T cells.[23]

Nevertheless, the precise pathways and molecules involved in PUVA-dependent T cell apoptosis remain largely unidentified.[24] Therefore, we reasoned that it would be important to gain more insight into the

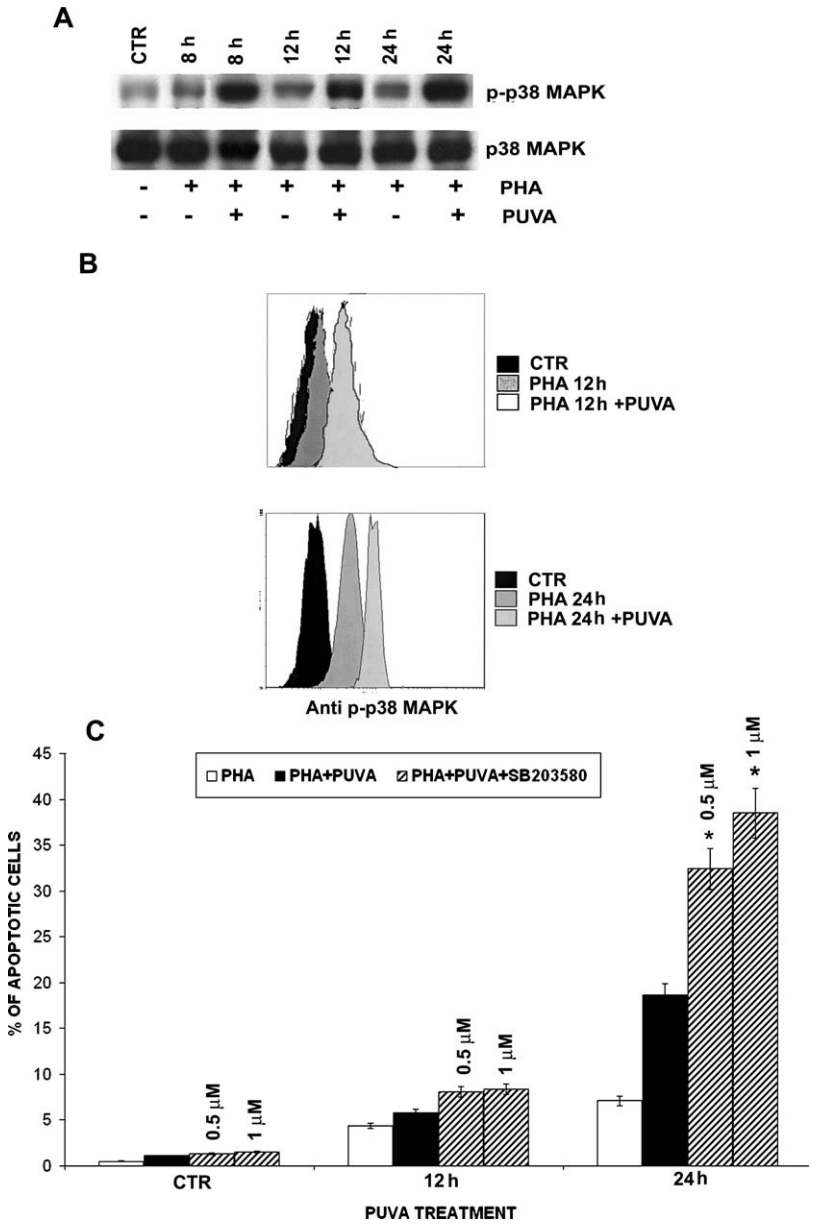
Figure 5. PUVA treatment does not activates Akt in Jurkat cells. A: western blot analysis for total Akt and its phosphorylated forms (Ser 473 and Thr 308) in Jurkat cells incubated up to 60 min after PUVA treatment. B: western blot analysis for total Akt and its phosphorylated forms in Jurkat cells incubated up to 16 h after PUVA treatment. SB203580 (10 μM) had been present since 45 min prior to PUVA exposure. C: western blot analysis for Thr 308 p-Akt in cells treated up to 16 h with LY294002 (25 μM). In A-C 80 μg of protein was blotted to each lane. Immunoreactive bands were visualized by the ECL technique. Immunostaining with a monoclonal antibody to β-tubulin confirmed equal loading in C. Blots are representative of three separate experiments. D: effects of SB203580 on the cell cycle of control Jurkat cells. Cells were incubated for 24 h in the presence of increasing concentrations of SB203580. PI-stained samples were then analyzed by flow cytometry for the detection of apoptotic (sub-G1) cells. Representative histograms of three different experiments are shown.



molecular mechanisms which underlay apoptotic cell death mediated by PUVA, because they might provide a way to modulate the effectiveness of PUVA and its clinical outcome. In this article, we have investigated whether or not PUVA treatment activates elements of the MAPK family in Jurkat human T lymphoblastoid cells and T lymphocytes from healthy donors. We have very recently shown that Jurkat T cells behave exactly as normal human T lymphocytes as far as PUVA-elicited caspase activation pattern was concerned[9] and for this reason they may be considered to represent a valid model to study apoptotic events associated with PUVA exposure.

Our findings demonstrated that neither p42/44 MAPK nor SAPK/JNK were activated following PUVA treatment of Jurkat cells. In contrast, we detected a rapid activation of p38 MAPK. It should be emphasized that activation of p38 MAPK was detected only when Jurkat cells were exposed to PUVA and not to either 8-MOP or UV-A alone. Indeed, it has been demonstrated that both SAPK/JNK and p38 MAPK could be rapidly activated by UV irradiation (UV-C) in Jurkat cells.[25] On the other hand, our findings clearly indicated that neither 8-MOP not UV-A alone were capable of inducing apoptosis of Jurkat cells, as demonstrated by flow cytometric analysis of PI-stained samples and of $\Delta\psi_m$.

Figure 6. PUVA treatment activates p38 MAPK in human T lymphocytes from healthy donors. A: Western blot analysis for p38MAPK (total and phosphorylated) performed on cell extracts prepared from cells exposed to PUVA and incubated up to 24 h. PHA concentration in culture medium was 0.5 μg/ml. A blot representative of three different experiments is shown. B: T lymphocytes treated with PUVA were fixed, permeabilized, and stained with an Alexa Fluor 488-conjugated antibody which recognizes p-p38 MAPK. Samples were analyzed by flow cytometry. Representative histograms of three different experiments are shown. C: SB203580 had been present since 45 min prior to PUVA exposure. Cells were incubated up to 24 h after treatment with PUVA. Results are the mean of three different experiments $\pm$ sd. The asterisk indicates significant differences ($p < 0.01$) with respect to samples not treated with SB203580. In A–C CTR: control (untreated) cells.



The relevance of p38 MAPK activation to the apoptotic process was demonstrated by the use of a pharmacological inhibitor of p38 MAPK, SB203580. Surprisingly, the drug was capable of significantly enhancing Jurkat cells apoptosis following exposure to PUVA. We also verified that SB203580 did not increase apoptosis by interfering with the survival pathway controlled by Akt.[26] Indeed, there is a report showing that SB203580, when employed at concentrations in excess of 2 μM, could inhibit Akt phosphorylation.[18] However, our results clearly showed that even a 0.5 μM SB203580 concentration resulted in significant apoptosis increase in response to PUVA treatment of Jurkat cells. Furthermore, it has been reported that UV-A induces Akt phosphorylation.[27] Nevertheless,

we have established that there was no activation of Akt in response to PUVA treatment, that SB203580 did not affect the phosphorylation levels of Akt, and that SB203580 only induced apoptosis in PUVA treated Jurkat cells, whereas it had no effect on control (untreated) cells.

We have also shown that an activation of p38MAPK was detectable in T lymphocytes from healthy donors exposed to PUVA. However, activation was slower in that it was at first detectable after 8 h since PUVA exposure. This is consistent with the slower apoptotic induction which characterizes T lymphocytes when compared with Jurkat cells.[9] In any case, SB203580 was capable of increasing in a significant manner the apoptosis rate also in T lymphocytes.

It should be pointed out that we detected an activation of p38 MAPK also in lymphocytes treated with PHA alone, even though it was less marked than in response to PUVA+PHA. Nevertheless, our unpublished results have shown that this activity was not related to cell survival because treatment with SB203580 did not increase apoptosis nor decreased proliferation in T lymphocytes exposed to PHA only. p38 MAPK activation has been previously reported in T lymphocytes exposed to proliferative stimuli including activation of the T cell receptor.[28] Also in this case, however, SB203580 failed to decrease proliferation but rather resulted in a reduced production of interleukin-5. PUVA treatment resulted in the disappearing of cells in the G2/M phase of the cell cycle. Conceivably, this depend on the fact that G2/M phase cells are the most sensitive to the action of PUVA and enter apoptosis. The increased sensitivity of G2/M cells to apoptotic stimuli has been previously reported.[29]

p38 MAPK activation is usually linked with apoptosis elicited by various stimuli, including heat shock, TNF-α, UV irradiation, and cytochalasin D.[30] However, p38 MAPK-dependent signaling pathways are involved in a variety of cellular responses, and the outcomes of these responses are varied and complicated. Indeed, it has been shown that in some cases p38 MAPK could enhance survival.[31,32] Most likely, this function is strictly dependent on cell and/or stimulus type. Conceivably, p38 MAPK activation is essential for the induction of target genes that prevent apoptosis of PUVA-treated cells. In Jurkat cells p38 MAPK up-regulates genes such as c-jun, egr-1, and C-terminal Src kinase Csk.[33] It is very intriguing that c-jun has been related to survival in Jurkat cells or T lymphocytes,[34] while egr-1 plays an antiapoptotic role in B cell lymphoma.[35] Also Csk has been shown to be involved in cell survival.[36] Therefore, in the future it will be very interesting to analyze what genes are activated in response to PUVA treatment of Jurkat cells and human T lymphocytes and to determine if their up-regulation is dependent on p38 MAPK. However, our preliminary results have indicated that c-jun does not appear to be up-regulated in PUVA-treated Jurkat cells (data not shown).

Another area of interest will be the elucidation of the activating pathways of p38 MAPK in response to PUVA. Indeed, p38 MAPK is phosphorylated and activated by dual kinases MKK3 and MKK6 which are under the control of up-stream kinases such as ASK1, MEKKs, and Tak1.[30]

In bone marrow transplantation (BMT) 30–60% of allogeneic BMTs are affected by chronic GvHD.[37,38] The effectiveness of PUVA therapy in cutaneous GvHD and in autoimmune disorders involving the skin has been shown and this type of treatment is effective also in patients with resistance to conventional immunosuppressive therapy[39] or where treatment was poorly tolerated.[40] Even though the relative contribution of apoptosis to the therapeutic effect of PUVA remains to be fully elucidated, we feel it might be very important to have tools which can increase the number of apoptotic cells in response to PUVA, thus possibly enhancing the tolerogenic response. Indeed, the sensitizing drugs might easily be administered to lymphocytes removed form patients and then washed out prior to re-infusion. This would not result in systemic toxicity by the chemicals and might improve the therapeutic outcome.

Conclusion

In this study we found that PUVA treatment of Jurkat cells and human T lymphocytes activates the p38 MAPK pathway but not the p42/44 MAPK or the SAPK/JNK signalling networks. The use of a pharmacological inhibitor selective for the p38 MAPK pathway, SB203580, allowed us to demonstrate that this network has an anti-apoptotic function in Jurkat cells and T lymphocytes from healthy donors in response to PUVA treatment. Moreover, we have shown that the effect of SB203580 was not due to a down-regulation of the Akt survival pathway which was not activated in response to PUVA. Our findings suggest that p38 MAPK-dependent signalling might be very important for the regulation of survival genes after exposure to PUVA. Since the therapeutic effect of PUVA seems to depend, at least in part, on apoptosis, further studies on the apoptosis signaling networks up-regulated by this treatment might lead to the use of signal transduction modulators in combination with PUVA, to increase the clinical efficacy of this form of therapy.

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