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Induction of interleukin 6 by ionizing radiation in a human epithelial cell line: control by corticosteroids

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Abstract. The cutaneous radiation syndrome after therapeutic or accidental exposure of human skin to ionizing radiation (IR) is accompanied by inflammatory processes which are controlled partly by proinflammatory cytokines. Besides tumour necrosis factor (TNF) — α and interleukin (IL)1, the pluripotent cytokine IL-6 belongs to the key mediators of inflammation. So far, there are no reports about the regulation of IL-6 by IR in epidermal cells. As an *in vitro* model to study the effects of IR on IL-6 gene expression, we treated the human epithelial HeLa cell line with different single X-ray doses between 1 and 20 Gy. Twenty-four hours after irradiation the IL-6 secretion was dose-dependently enhanced as measured by ELISA. At the transcriptional level, a slight increase of IL-6 transcripts was already detectable 1 h after irradiation, with maximum levels at 2 h, and a decline to baseline levels between 8 and 24 h. Addition of the transcriptional inhibitor actinomycin D inhibited the inducibility of IL-6 mRNA by TPA and IR. As the IL-6 promoter contains multiple binding sites for activated glucocorticoid receptors within the 5′ regulatory region, the potential modulation of IL-6 expression by the corticosteroids hydrocortisone, dexamethasone and mometasone furoate was included in our study to modify the radiation-induced stress response. All corticosteroids applied could efficiently downregulate TPA- or radiation-induced IL-6 expression on both gene expression and protein levels. Mometasone furoate, followed by dexamethasone, was found to be most effective at low concentrations (1 nM), whereas hydrocortisone had to be applied at about 100-fold higher concentrations to achieve comparable inhibition. This experimental model is aimed at understanding the molecular circuits following IR, and thus to provide a basis for the treatment of radiation effects in skin.

1. Introduction

The cutaneous radiation syndrome (Peter *et al.* 1994) after therapeutic or accidental exposure of skin to ionizing radiation (IR) is accompanied by the infiltration of immunologically competent cells like neutrophilic and eosinophilic granulocytes, T-cells and macrophages (Trott and Kummermehr 1991). Intercellular communication between these cells and the cells of the skin, such as keratinocytes and fibroblasts, is mediated partly by the secretion of proinflammatory cytokines (Kupper 1990). The understanding of the molecular regulation of genes induced by UV-radiation and other agents causing genotoxic stress is of particular interest (Weichselbaum *et al.* 1991, Keyse 1993, Herrlich and Rahmsdorf 1994).

Besides tumour necrosis factor (TNF)-α and IL-1, both of which are released by different cell types after IR (Hallahan *et al.* 1989, Sherman *et al.* 1991, Baker *et al.* 1995, Ishihara *et al.* 1995), the pluripotent cytokine IL-6 can mediate not only acute proinflammatory but also regenerative processes leading to immunological control and reconstitution of tissues. The various effects of IL-6 *in vivo*, which include induction of fever, acute-phase response, antibody formation as well as activation of natural killer cells and cytotoxic T-lymphocytes, indicate the importance of IL-6 as a key cytokine of the immune response (Akira *et al.* 1990, Van Snick 1990), Fattori *et al.* 1994, Chai *et al.* 1996). Furthermore, IL-6 and the radiation-inducible cytokine TNF-α were found to exert synergistic anti-tumour responses in mouse (Mule *et al.* 1990, 1992). The observation of a strong acute-phase reaction of irradiated mouse (Hong *et al.* 1995, Magic *et al.* 1995) as well as the immediate induction of local erythema in the skin of therapeutically irradiated patients points to a role of IL-6 which is induced and secreted in high quantities early after oxidative injury of many cell types, including keratinocytes and fibroblasts (Urbanski *et al.* 1990, Kirnbauer *et al.* 1991, Brach *et al.* 1993, de Vos *et al.* 1994, Kick *et al.* 1995).

Ionizing radiation results in the formation of short-lived highly reactive oxygen intermediates (ROI), which are supposed to be the main mediators of intracellular damage, defined as the indirect radiation effect (Halliwell and Gutteridge 1985). The physiological role of ROI, which also can be produced in large amounts by granulocytes and macrophages directed against micro-organisms, is intracellularly defined as a second-messenger system leading to gene regulation, and thus to a broad spectrum of immuno-

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regulatory events. Electromagnetic waves like UV-radiation, IR, as well as reoxygenation after hypoxia, are oxidative stressors which involve this stress response pathway (Schreck *et al.* 1991, Baeuerle and Henkel 1994, Herrlich and Rahmsdorf 1994, Rupec and Baeuerle 1995). Intriguingly, as a molecular target at the level of gene regulation the transcription factor nuclear factor κB (NF-κB) plays a pivotal role in transducing the oxidative stress signal to the nucleus.

As an *in vitro* model to study the effects of IR on IL-6 gene expression, we used the epithelial HeLa cell line, which is known to produce large amounts of this cytokine after activation with phorbol esters and other stimuli (Ray *et al.* 1989, Kick *et al.* 1995). In view of this, we wanted to investigate the potential of IR to induce IL-6 gene expression and to explore the intracellular regulatory steps of early induced genes and immunomodulatory pathways.

Although IL-6 can initiate anti-tumoral activity in concert with other cytokines, the radiation-induced inflammation has side-effects to the whole organism. There is at the present time no established therapy available to overcome the side-effects of tumour irradiation. This points to the aim to reduce the detrimental systemic influences of uncontrolled inflammatory cascades without affecting the capability of the irradiated healthy tissue to proliferate and heal in regard to tissue remodelling after tumour therapy. The widely used corticosteroids can be applied in the treatment of inflammatory diseases with synthesis of IL-6 like acute-phase reaction, sepsis, psoriasis vulgaris, autoimmune diseases, or after exposure to ultraviolet (UV) light (Akira *et al.* 1990, Urbanski *et al.* 1990, Van Snick 1990, Linker Israeli *et al.* 1991, Neuner *et al.* 1991). The mechanisms of the beneficial action of corticosteroids are supposed to be partly due to an inhibition of cytokine gene expression. IL-6 can be downregulated efficiently by corticosteroids (Ray *et al.* 1990, Barton *et al.* 1991, Levine *et al.* 1993). While the negative regulation of IL-6 has been identified for numerous stimuli like lipopolysaccharide and IL-1 by corticosteroids in different cell lines (Ray *et al.* 1989, Waage *et al.* 1990), so far nothing is known about the mechanisms involved in the downregulation of IR-induced IL-6 activation. On the molecular basis it is known that the IL-6 promoter contains at least four DNA recognition sites for activated glucocorticoid receptors (GRE) (Ray *et al.* 1990), which suggests a strong inhibitory capacity of corticosteroids on the expression of this potent proinflammatory cytokine.

In the case of patients undergoing radiotherapy or suffering from accidental exposure to IR, the use of corticosteroids is controversial because of their anti-proliferative effects potentially leading to clonal depression and epidermal atrophy. Nevertheless, the beneficial action of systemically applied corticosteroids as treatment for the cutaneous radiation syndrome has been described previously (Chung *et al.* 1972). We used the derivatives dexamethasone and mometasone furoate and compared the capacity in modifying the IR-induced stress response *in vitro* to hydrocortisone. As an experimental model on the cellular basis their influence on IL-6 mRNA expression and protein synthesis was examined.

We report on induction of IL-6 expression by IR in a time- and dose-dependent manner upon irradiation of HeLa cells by X-rays. The observed upregulation of IL-6 on both the transcriptional and protein level could be inhibited in a dose-dependent fashion by the three corticosteroid derivatives applied.

2. Materials and methods

2.1. *Chemicals*

12-*O*-tetradecanoyl phorbol 13-acetate (TPA), hydrocortisone (H) and dexamethasone (D) were obtained from Sigma Chemical Co. (Deisenhofen, Germany). Mometasone furoate (M) was kindly provided by Essex Pharma GmbH (Munich, Germany).

2.2. *Cell culture and treatment*

HeLa cells were grown in DMEM culture medium supplemented with 10% heat inactivated foetal calf serum (FCS, Boehringer Mannheim, Mannheim, Germany). Cells were trypsinized and 3×10^6 cells were seeded per 10-cm petri dish. Twenty-four hours later culture medium was replaced by DMEM containing 0·5% FCS. A further 24 h later the subconfluent cells were treated with TPA (100 ng/ml) or irradiated with X-rays (240 kV, 13 mA, FOD 40 cm, filter 2·0 mm Cu) at a dose rate of approximately 1 Gy/min. Viability of the cells was confirmed for each single dose applied by the trypan blue exclusion method with at least 70% surviving cells 24 h after 20 Gy.

2.3. *IL-6 ELISA*

Levels of IL-6 protein in cell free supernatants of HeLa cells were determined 24 h after treatment with TPA, IR and cotreatment by corticosteroids using an ELISA kit (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) according to the manufacturers' advice.

2.4. *Northern blot analysis*

Total RNA was extracted using the RNA Clean kit (AGS, Heidelberg, Germany) according to the manufacturers' protocol, which is based on the single step guanidinium isothiocyanate method (Chomczynski and Sacchi 1987). Of total RNA, 15–20 μg was denatured by heating to 50°C for 30 min in a buffer containing glyoxal and DMSO. After size fractionation on a 1%-agarose gel, RNA was transferred to a Hybond-N membrane (Amersham-Buchler, Braunschweig, Germany) and hybridized overnight under stringent conditions at 42°C in a solution containing 1 M sodium chloride, 10% dextrane sulphate, 50% formamide and 1% sodium dodecyl sulphate. All blots were sequentially hybridized with cDNA probes encompassing 300 bp of the human IL-6 and 1·1 kb of the human β-actin gene, both labelled by random nonamer priming using the Megaprime kit (Amersham-Buchler) by use of [^{32}P]dCTP (NEN DuPont, Dreieich, Germany). The blots were exposed for 1 day to radiographic films (Kodak X-OMAT AR) at -70°C to detect a specific 1·4 kb (IL-6) or 1·8 kb (β-actin) band.

3. Results

3.1. *X-ray-induced secretion of IL-6*

To examine whether IL-6 production can be enhanced by ionizing radiation, experiments using culture supernatants of human epithelial HeLa cells were carried out. Increased IL-6 protein levels were measured by ELISA 24 h following IR compared with unirradiated controls (data not shown). To determine the dose-dependence of this effect, HeLa cells were irradiated with increasing single doses (1–20 Gy). As shown in Figure 1, a clear dose-dependent increase in IL-6 secretion levels up to a single dose of 20 Gy was observed.

3.2. *Time and dose-dependency of X-ray-induced IL-6 mRNA levels*

To elucidate further the molecular regulation of IL-6 induction Northern blot analyses were carried out with an IL-6-specific cDNA probe which detected a 1·4 kb transcript after hybridization. For determination of IL-6 transcript levels the stimulated samples were harvested at different points in time after the application of a single dose of 12 Gy. As positive control the cells were treated with TPA (100 ng/ml) in parallel (Figure 2, lane 1). Low constitutive IL-6 mRNA levels of control cells harvested immediately after irradiation (lane 2) could be observed. A slight increase was already detectable 1 h post-irradiation

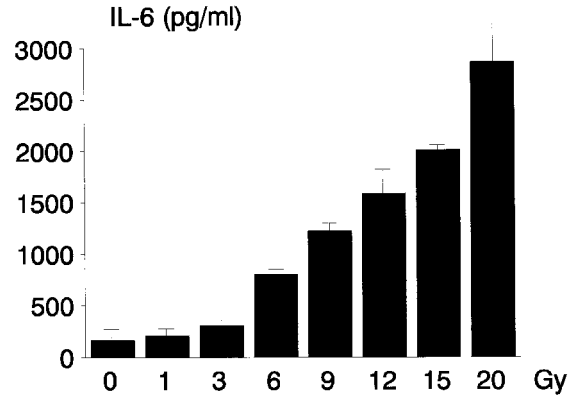

Figure 1. X-ray-induced secretion of IL-6 in HeLa cells. Cultured cells were irradiated with the indicated single doses (1–20 Gy). After 24 h cell free supernatants were prepared to measure IL-6 protein levels by ELISA. Data represent the median of three independent experiments. The standard error of the mean is indicated. All data except the 1 Gy bar have $p<0{\cdot}05$ compared with the unirradiated control.

(lane 3). Stronger accumulation of mRNA signals appeared between 2 and 4 h and maximum levels were reached approximately 2 h after irradiation (lane 4). Decline to base line levels occurred between 3 and 8 h (lanes 5–8).

As the maximal increase of IL-6 expression was measured 2–4 h post-radiation in cellular systems (Figure 2) (Brach *et al.* 1993), the following dose–response kinetics were performed. We applied 3–20 Gy single doses to investigate the effect on IL-6 mRNA accumulation. A strong dose-dependent increase of IL-6 transcript levels starting from 3 Gy was observed (Figure 3, lanes 2–7). These results were in good agreement with our data on IL-6 protein secretion (Figure 1).

3.3. *IR-enhanced IL-6 transcript levels by transcriptional activation*

For a more detailed analysis of IL-6 induction, cells were incubated in the presence or absence of the transcriptional inhibitor actinomycin D (act D, 5 μg/ml) 1 h prior to irradiation with a single dose of 12 Gy. HeLa cells cotreated by act D and TPA did not show increased transcript levels compared with the low constitutive expression of IL-6 (Figure 3, lane 1 and Figure 4, lane 2). Cells treated with X-rays alone (Figure 4, lane 3) showed a clear induction of IL-6 transcripts and, furthermore, act D pretreatment could effectively counteract the induced expression followed IR (lanes 3 and 4). Similar results were obtained from cells treated with TPA and act D (lanes 1 and 2), reflecting that transcriptional

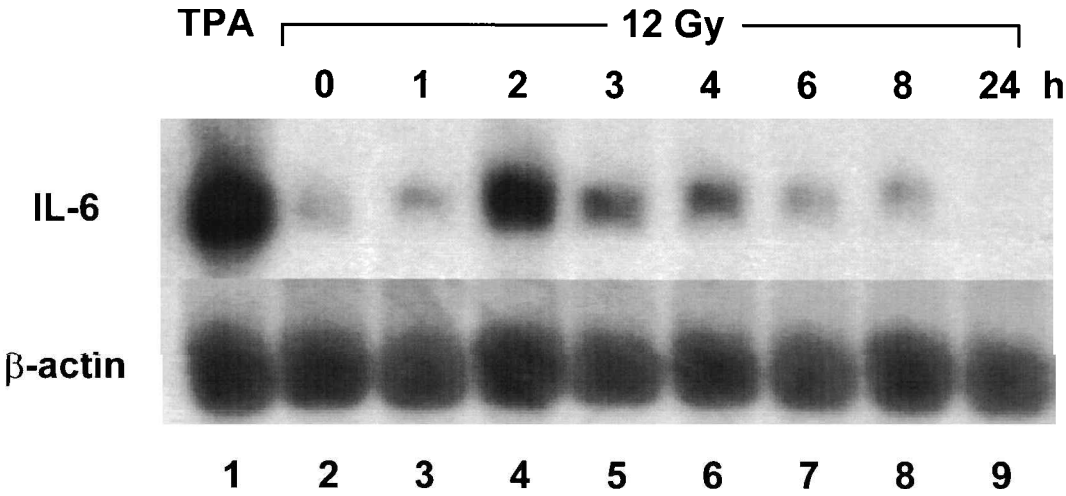

Figure 2. Time-dependence of IL-6 transcript accumulation. Total RNA (20 μg) was extracted at the indicated time points after irradiation with 12 Gy (240 kV, 13 mA) (lanes 2–9), or after treatment with TPA (100 ng/ml) for 2 h (lane 1) and size-separated by gel electrophoresis. Blots were hybridized to a ^{32}P-labelled specific human IL-6 cDNA fragment. Rehybridization with a specific human 'β'-actin cDNA was performed to confirm comparable RNA-loading. Transcripts were visualized by autoradiography. One of three experiments is shown.

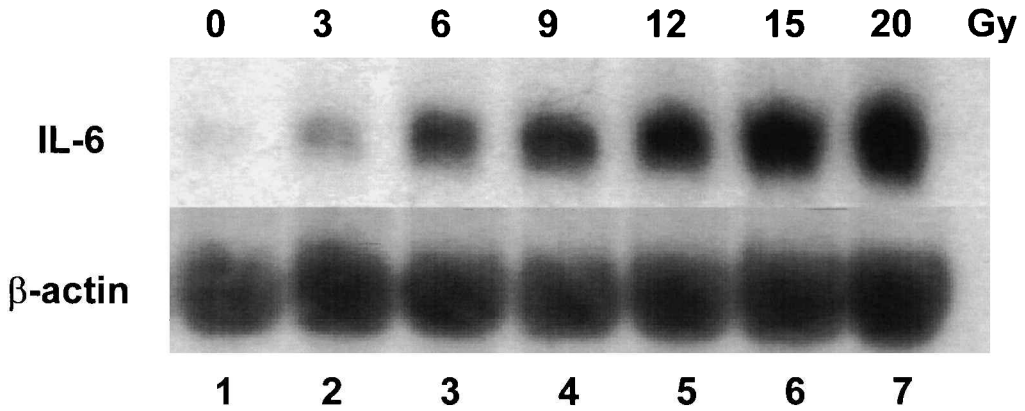

Figure 3. Dose–response of IL-6 transcript accumulation after IR. HeLa cells were grown in medium without stimulation (lane 1) or irradiated with the indicated single doses of X-rays (lanes 2–7). Total RNA was extracted 2 h after irradiation. Data represent one of three essentially identical experiments.

induction is responsible for the accumulation of IL-6 transcripts by both TPA and X-ray treatment.

3.4. *Downregulation of TPA- and IR-induced IL-6 protein and transcript levels by corticosteroids*

To investigate the effects of the corticosteroid derivatives hydrocortisone, dexamethasone and mometasone furoate on IL-6 secretion levels, the three steroids were applied 1 h before treatment with TPA (100 ng/ml) in concentrations ranging from 1 to 100 nM and IL-6 protein levels were examined by ELISA 24 h thereafter (Figure 5). As the mechanism of efficient inhibition of TPA-induced IL-6 transcriptional expression by dexamethasone in HeLa cells was previously studied in a model applying cotransfection of exogenous glucocorticoid receptors (Ray *et al.* 1990), we were particularly interested in investigating the effects of the three derivatives under non-optimized but physiological conditions. Indeed, the strong induction of IL-6 protein by TPA was markedly reduced in steroid treated cells (Figure 5). Dexamethasone (D) and mometasone furoate (M) were already effective at low concentrations of 1 nM, whereas for hydrocortisone (H) about a 100-fold higher concentration (100 nM) was needed to achieve equal repression of IL-6 protein levels. Among these three steroids hydrocortisone appeared to confer the weakest inhibition of IL-6.

To elucidate whether the observed inhibition of IL-6 protein expression is paralleled by downregulation of mRNA levels, Northern blot analyses were

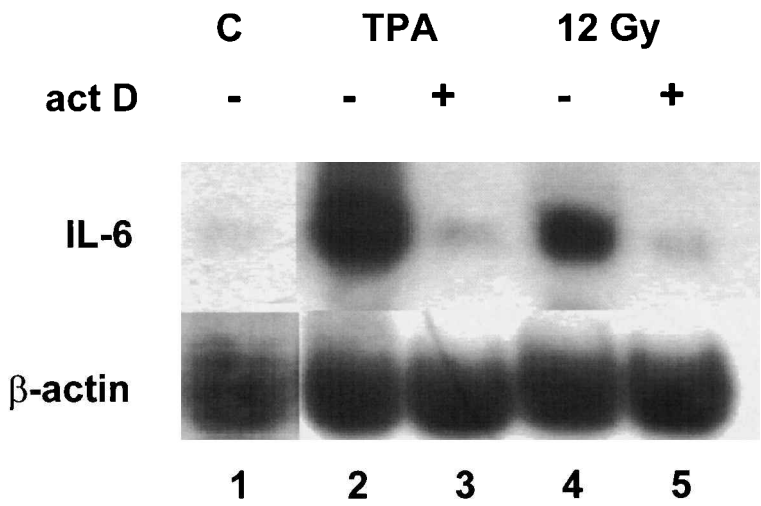

Figure 4. Transcriptional activation of IL-6 transcript accumulation. HeLa cells were either treated with TPA (lanes 2 and 3) or irradiated with 12 Gy (lanes 4 and 5). Untreated controls (C) are shown in lane 1. In lanes 3 and 5 actinomycin D (act D, 5 μg/ml) was added 1 h before stimulation.

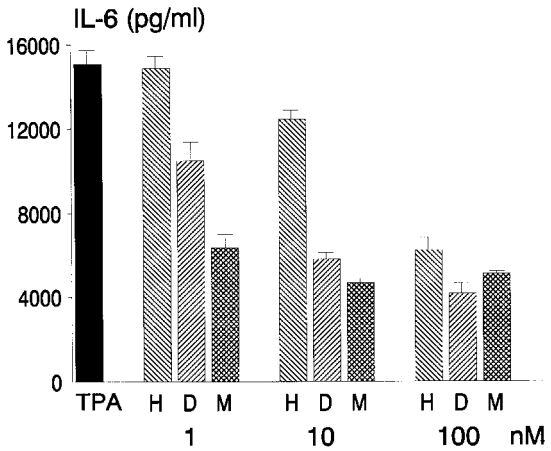

Figure 5. Inhibition of TPA-induced IL-6 protein secretion by corticosteroids. HeLa cells were stimulated with TPA (100 ng/ml). Cells were pretreated with varying concentrations between 1 and 100 nM hydrocortisone (H), dexamethasone (D) or mometasone furoate (M) 1 h before stimulation. After 24 h IL-6 protein levels were measured by ELISA. Data represent the median of three independent experiments. The standard error of the mean is indicated. All data except the bars for 1 and 10 nM treatment with hydrocortisone have $p<0{\cdot}05$ compared with the TPA-treated control.

carried out (Figure 6). Total RNA was extracted 2 h after treatment with TPA or IR, as for both modalities maximal mRNA expression was found (Kick *et al.* 1995 and Figure 2 respectively). According to the data obtained at the protein level, we detected reduced amounts of IL-6 transcripts for those samples which were incubated with one of the corticosteroids before TPA treatment (Figure 6, lanes 3–11). Dexamethasone and mometasone furoate were efficient, starting at a dose of 1 nM, whereas for hydrocortisone a concentration of 100 nM was needed to obtain similar downregulation of IL-6 mRNA levels (compare lanes 3 and 9). According to the ELISA data presented, dexamethasone and mometasone furoate reached maximal effectivity at a 10 nM concentration (Figure 5 and 6).

The IL-6 expression by X-rays was analysed using the same conditions as reported for TPA and corticosteroid cotreatment (Figures 7 and 8). At the level of protein synthesis at a concentration of 1 nM the compound mometasone furoate was clearly most effective in reducing the IL-6 protein levels from 1870 to 190 pg/ml compared with 740 pg/ml by dexamethasone and 1470 pg/ml by hydrocortisone. At a 100-fold higher inhibitory concentration (100 nM) hydrocortisone reduced IL-6 protein levels to 340 pg/ml. Thus, the efficacy was described as before for TPA and corticosteroid cotreatment ($M > D > H$). The corticosteroid mometasone furoate led to the strongest decrease of IR-induced IL-6 protein levels at all concentrations tested (Figure 7). By Northern blot analysis, again mometasone was the most effective drug, followed by dexamethasone and hydrocortisone (Figure 8).

4. Discussion

In this study, we show that IR is a potent inducer of IL-6 protein synthesis in the epithelial cell line HeLa and compare the dose–response and kinetics of IR with TPA treatment. We have found significantly increased IL-6 levels starting at doses of 3–20 Gy, which are accompanied by accumulation of IL-6 transcripts from 1 to 4 h after irradiation. The upregulation of both transcriptional activity and protein

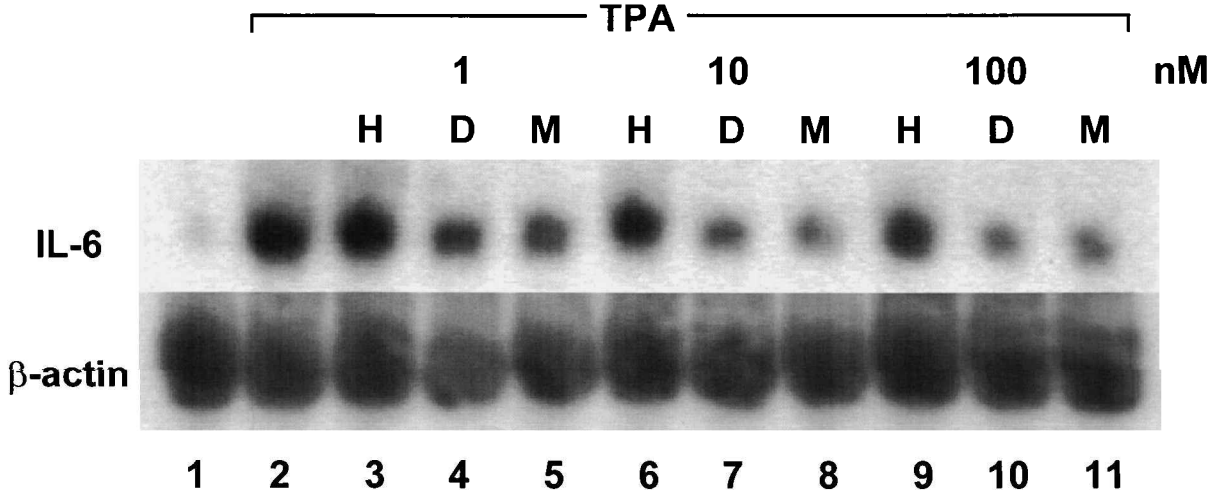

Figure 6. Inhibition of TPA-enhanced IL-6 transcript accumulation by corticosteroids. Cells were treated for 2 h with TPA (lanes 2–11) or left untreated (lane 1). Cells were pretreated with varying concentrations between 1 and 100 nM hydrocortisone (H; lanes 3, 6 and 9), dexamethasone (D; lanes 4, 7 and 10) or mometasone furoate (M; lanes 5, 8 and 11) respectively. Data represent one of two experiments.

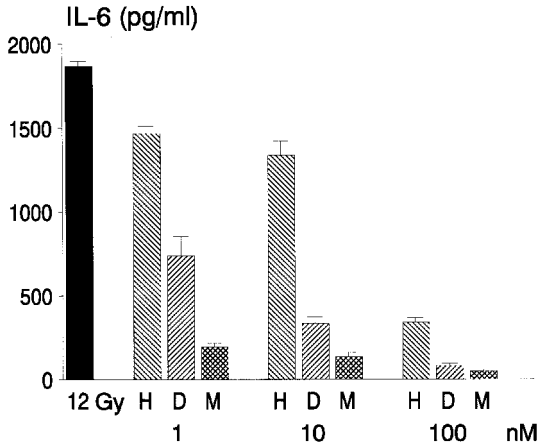

Figure 7. Inhibition of X-ray-induced IL-6 protein secretion by corticosteroids. HeLa cells were stimulated by a single dose of 12 Gy. One hour prior to irradiation cells were pretreated with varying concentrations between 1 and 100 nM hydrocortisone (H), dexamethasone (D) or mometasone furoate (M) respectively. After 24 h IL-6 protein levels were measured by ELISA. Data represent median of three independent experiments. The standard error of the mean is indicated. All data have $p<0{\cdot}01$ compared with the irradiated control.

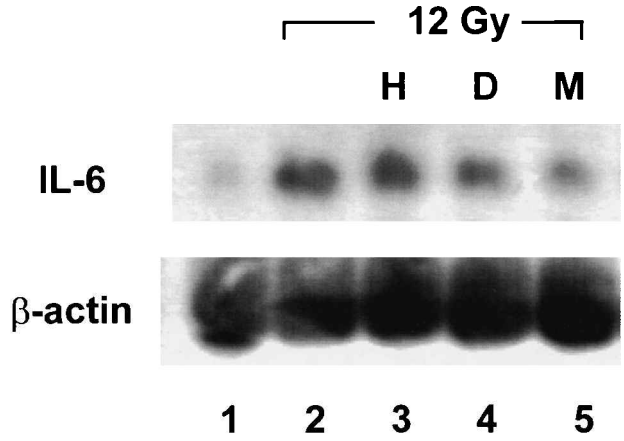

Figure 8. Inhibition of irradiation-enhanced IL-6 transcript accumulation by corticosteroids. Cells were irradiated with 12 Gy (lanes 2–5) or left untreated (lane 1) and total RNA was extracted after 2 h. Cells were pretreated with the effective concentration of 1 nM hydrocortisone (H; lane 3), dexamethasone (D; lane 4) or mometasone furoate (M; lane 5), respectively. Data represent one of two essentially identical experiments.

production levels by X-rays and TPA could be counteracted by use of the immunosuppressants hydrocortisone, dexamethasone and mometasone furoate. Our findings are in accordance with other groups, who described the induction of IL-6 in different cell types, including fibroblasts and keratinocytes, by the spectrum of electromagnetic waves like UVA, UVB or IR (Urbanski *et al.* 1990, Kirnbauer *et al.* 1991, Brach *et al.* 1993, Wlaschek *et al.* 1993, de Vos *et al.* 1994, Kick *et al.* 1995, Chung *et al.* 1996).

In UV-irradiated HeLa cells maximal IL-6 mRNA accumulation was observed about 4–8 h post-radiation, preceded by enhanced AP-1 activity, indicating transcriptional induction (Kick *et al.* 1995, 1996). A similar time course was described for UV-irradiated cultured primary keratinocytes with maximal IL-6 mRNA levels 6 h after treatment.

IL-6 regulation has been studied in various cell lines. Previously published data clearly showed

transcriptional initiation upon multiple physiological stimuli, like the cytokines IL-1α (Ray *et al.* 1989, Shimizu *et al.* 1990), IL-1β (Brach *et al.* 1993), TNF-α (Libermann and Baltimore 1990, Zhang *et al.* 1990), and viral infection with HTLV-1 (Yamashita *et al.* 1994), HIV-1 (Iwamoto *et al.* 1994) and rhinovirus (Zhu *et al.* 1996). The well-investigated IL-6 promoter contains DNA-binding sites for *cis*-activating transcription factors including NF — κB, activator protein (AP)-1, AP-2, nuclear factor IL-6 (NF-IL-6/CEBPβ) and cAMP-responsive element binding protein (CREB) (Libermann and Baltimore 1990, Ray *et al.* 1989, 1990, Shimizu *et al.* 1990, Zhang *et al.* 1990, Margulies and Sehgal 1993), three of which, NF-κB, AP-1 and CREB were reported to be activated by IR (Brach *et al.* 1993, Hallahan *et al.* 1993, Sahijdak *et al.* 1994).

Our data obtained from the well established cellular system of the human HeLa cell line suggest mainly the contribution of transcriptional initiation to the transient upregulation of IL-6 mRNA levels. At a single dose of 12 Gy, IL-6-specific mRNA levels could not be induced when the cells were incubated with actinomycin D prior to irradiation.

Transcriptional initiation after exposure to IR was also observed in human skin fibroblast lines, and no activation of the IL-6 promoter could be measured after truncation of the NF-κB recognition sequence identifying this transcription factor to be involved in the oxidative stress signal transduction (Brach *et al.* 1993).

In addition, AP-1 was shown to be activated upon X-irradiation in human tumour cell lines (Hallahan *et al.* 1993). In HeLa cells, increased AP-1 activity was found after UV-irradiation and photodynamic treatment, followed by transiently upregulated IL-6 transcript levels (Kick *et al.* 1995). In protein extracts from human melanoma cells CREB was found to be activated following exposure to IR (Sahijdak *et al.* 1994), but there are no reports about a correlation between IR-mediated CREB activation and IL-6 induction so far. Thus NF-κB, AP-1 and CREB seem to be the primary candidates for IR-mediated transcriptional induction. It is not quite clear, however, whether other not IR-responsive proteins bind to X-ray-responsive elements of the IL-6 promoter, as shown for example for the induction of the t-PA promoter in human melanoma cells (Boothman *et al.* 1994).

As the increase of IL-6 mRNA levels occurred in our system as early as 1–2 h after IR, the underlying mechanisms may be different from other treatments described before. Northern blot analyses carried out with the same HeLa cells showed increased IL-6 mRNA signals after 30 min to 2 h upon TPA stimulation (Kick *et al.* 1995). Compared with treatment by UV-light and photodynamic therapy, for the latter photofrin is used as photosensitizer and long-wave monochromatic light (630 nm), both TPA stimulation and IR seem to be more rapid inducers of IL-6 mRNA (compare Kick *et al.* 1995 and Figure 2).

The established model of cutaneous inflammation starts with the secretion of preformed and newly synthesized IL-1 and synthesis of TNF-α by activated keratinocytes inducing paracrine and autocrine mechanism of cytokine activation and secondarily the production of cytokines (Kupper 1990, Bos and Kapsenberg 1993). As both, IL-1 and TNF-α have the capacity to induce IL-6, and both are induced after IR (Hallahan *et al.* 1989), Sherman *et al.* 1991, Baker *et al.* 1995, Ishihara *et al.* 1995), the possibility exists that the IL-6 induction by IR could be a secondary effect of activation of the primary cytokines IL-1 and TNF-α. Interestingly, evidence was shown for this mechanism to be involved in cytokine production of keratinocytes exposed to UVB-radiation. IL-6 induction occurred 6 h after UV treatment and was downregulated by addition of anti-IL-1 antibodies (Chung *et al.* 1996). However, as the maximal increase of IL-6 expression in our system was observed as early as 2 h after IR and no biphasic response was detected, it seems very unlikely that increased IL-6 transcription could be caused by earlier expressed cytokines. Previously it was suggested that the gene regulatory effects of IR are caused by DNA damage. Alterations at the cell membrane are also proposed as one possible initiating step in the cellular radiation response (Haimovitz Friedman *et al.* 1994, Schieven and Ledbetter 1994). It was demonstrated that IR can act directly on membrane preparations devoid of nuclei leading to sphingomyelin hydrolysis and the formation of ceramide (Haimovitz Friedman *et al.* 1994). This observation resembles previously published work from other groups, using UV-light as an oxidizing agent, which described particularly the early signal transduction via receptor or non-receptor tyrosine kinases. In HeLa cells, pp60-Src or epidermal growth factor (EGF) receptor were phosphorylated during the first minutes after UV-radiation, which was followed by the sequential activation of *ras*, *raf* and downstream acting MAP-kinase-like kinases, leading to phosphorylation of *jun* (Devary *et al.* 1992, Sachsenmaier *et al.* 1994). In agreement, IR could mimic the EGF-mediated phosphorylation of the EGF receptor (Laderoute *et al.* 1994, Schmidt-Ullrich *et al.* 1996). Activation of the Src-like p56/p53 *lyn* tyrosine kinase in HL-60 myeloid leukaemia cells was found after IR and a direct interaction with cell cycle control proteins could be shown (Kharbanda *et al.* 1994).

Taking these reports together with the fact that the two potential ligands of the EGF receptor, EGF and TGF-α were found to induce the synthesis of IL-6 in thymic epithelial cells (Le *et al.* 1991), it seems possible that the observed IL-6 induction by IR could at least partly be regulated by early involvement of tyrosine kinases. We have observed increased expression of EGF receptors in human keratinocytes as well as in biopsies from patients undergoing radiotherapy (Peter *et al.* 1993).

To overcome the detrimental effects of an uncontrolled rise of proinflammatory cytokines as an acute response to IR, therapeutic drugs capable of inhibiting the pathways leading to transcriptional activation of cytokine genes are of particular interest. We describe here the potency of hydrocortisone, dexamethasone and mometasone furoate to inhibit radiation-induced IL-6 expression. Mometasone furoate and dexamethasone were shown to be much more effective than hydrocortisone. This result correlates with previously reported experiments where IL-6 (Barton *et al.* 1991, Levine *et al.* 1993) as well as TNF-α and IL-8 induction (Michel *et al.* 1995) were inhibited in epithelial and leukaemic cells by corticosteroids.

In the past few years knowledge about how corticosteroids exert their modulatory activity upon transcription has increased dramatically. Corticosteroids bind intracellular receptors (GR), which enable the complex to translocate into the nucleus and bind to cognate glucorticoid responsive elements (GRE). Three of the previously described four GRE of the IL-6 promoter are located in positions of transcription factor binding motifs, like the multiple response element (MRE) containing the cAMP-responsive element (CRE), the TPA-responsive element (TRE) and the NF-IL-6/CEBPβ site, and furthermore the basal transcriptional units like the TATA-box and the initiation site of transcription (Ray *et al.* 1990). It is conceivable that the bound GR inhibit the recognition of transcription factors involved in *cis*-activation of the IL-6 promoter.

More recently, it was demonstrated that the action of corticosteroids on transcriptional regulation can be complex. For example, an inhibitory effect of corticosteroids was also described for genes without functional binding sites or consensus-like sequences for activated GR. In this regard evidence was provided for a physical association of the ligand-bound GR with the p65 subunit of NF-κB (Ray and Prefontaine 1994b, Scheinman *et al.* 1995b) or with AP-1-family proteins (Yang Yen *et al.* 1990, König *et al.* 1992). In addition, it was shown that GR are able to inhibit the translocation of the activated form of NF-κB from the cytoplasm into the nucleus (Scheinman *et al.* 1995b). This second mechanism would efficiently inhibit the binding of activating transcription factors to the consensus elements of multiple promoters and block the initiation of transcription of immunoregulatory genes. Finally, it was shown by two groups that glucocorticoids can activate transcription and expression of IκB-α, the physiological inhibitor protein of NF-κB, in the cytoplasm (Auphan *et al.* 1995, Scheinman *et al.* 1995). Due to corticosteroids, proteolytically degraded inhibitor would be immediately replaced by freshly synthesized IκB-α, which could then prevent the translocation of NF-κB to the nucleus.

At the present time it is not known whether the observed inhibitory effects of hydrocortisone, dexamethasone and mometasone furoate on IR-induced IL-6 activation in HeLa cells follow the mechanisms discussed. For future investigations the IR-sensitive epithelial HeLa cell line seems to be a promising *in vitro* model to get more insight in the differential steps of regulation by corticosteroids and their capacity to suppress cytokine production in regard to the treatment of individuals exposed accidentally or therapeutically to IR. Finally, the reported low atrophogenic potential of mometasone furoate after topical application (Belsito *et al.* 1988, Katz *et al.* 1989), together with the strongest capacity of IL-6 downregulation in IR-treated cells points to this drug as the most promising derivative of the three corticosteroids investigated.

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