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Radiation-induced alterations in cytokine production by skin cells

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Ionizing radiation exposure of skin results in a cutaneous radiation reaction comprising all pathophysiological reactions and clinical symptoms in irradiated skin. Biological responses of skin occur in a characteristic temporal pattern and mainly depend on radiation quality, dose rate, total dose, and cellular conditions. Immediately after irradiation, production of cytokines by skin cells is initiated and continues as a cascade during all stages of the cutaneous radiation syndrome leading to progressive late symptoms, the predominant of which is fibrosis. Cytokines are important signaling molecules mediating communicative interactions both locally between different cell types within dermal tissues and distantly between organs. Although during recent years much progress has been made in dissecting the complex cytokine network, the role of cytokines in the pathophysiology of the cutaneous radiation reaction is only beginning to be elucidated. Previous studies indicate that the major cytokines in the response of skin cells to ionizing radiation include IL (interleukin)-1, IL-6, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, and the chemokines IL-8 and eotaxin. In this paper, existing data on the radiation-induced modulation of cytokine expression by skin cells are reviewed. © **2007 International Society for Experimental Hematology. Published by Elsevier Inc.**

The pathophysiology of the cutaneous radiation reaction

The term cutaneous radiation reaction, also referred to as cutaneous radiation syndrome, describes the total of pathophysiological reactions and clinical symptoms induced in skin as a result of ionizing radiation exposure. Skin reactions occur in a characteristic temporal pattern consisting of prodomal erythema, manifestation stage, subacute stage, chronic stage, and late stage [1,2]. However, duration and severity of the individual phases, the degree of skin damage, and the ability of skin cells to recover from radiation damage mainly depend on radiation quality, dose rate, total dose, and cellular conditions [1–3]. Ionizing radiation may have an impact on virtually every single component of the cell. It not only affects the proliferative capacity of skin stem cells, but also modulates the communication between cells leading to skin damage and impaired cutaneous integrity. In the initial phase of the cutaneous radiation syndrome, a transient and inconsistent erythema may occur. In this early phase, a few hours after irradiation, a transcriptional activation of a cascade of cytokines is initiated. The cytokines released by irradiated cells bind to receptors on cells within the same tissue or on distant cells and stimulate them to generate a biological response [4]. Cytokines induce the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) on keratinocytes and endothelial cells as well as vascular cell adhesion molecule (VCAM) and E-selectin on endothelial cells [5–8]. This activation pattern causes an increased vascular permeability, activation of inflammatory cells, and their transendothelial migration from the circulation into the inflamed tissue. The onset and intensity of the early erythema are determined by the radiation dose. This condition is dose-dependent and clinically asymptomatic as long as a balance between proinflammatory and anti-inflammatory processes exists. Up to now it has been commonly accepted to distinguish between different clinical stages of the cutaneous radiation reaction. Although new data from a retrospective analysis of clinical courses of the cutaneous radiation reaction in radiation victims suggest that a strict separation of these individual clinical stages in general may no longer be reasonable (Meineke et al., manuscript in preparation), this old classification still bears some didactic advantages with respect to the understanding of the pathophysiological sequence of the events occurring in radiation-exposed skin. According to this classification, within days to a few weeks, the manifestation stage may appear. This stage is characterized by intense reddening, blistering, and ulceration of the irradiated

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tissue. Tissue destruction, capillaritis and vasculitis of dermal venules and arterioles, and granulocyte infiltration result in the development of a complex wound [9]. As a consequence of these inflammatory reactions, considerable tissue damage gradually evolves. In the chronic stage of the cutaneous radiation syndrome, with a latency of 3 months to 2 years, epidermal keratosis, atrophy, telangiectasia, and fibrosis may occur. Fibrosis is progressive by nature. Its predominant characteristics are massive deposition of extracellular matrix and excessive fibroblast proliferation [9–11].

Taken together, cytokine expression is initiated immediately after irradiation and persists for months and possibly years, having an impact on all stages of the cutaneous radiation syndrome. This review will summarize the current knowledge on radiation-induced modulation of cytokine expression in skin cells.

General characteristics of cytokines

Cytokines are soluble polypeptides that are produced by a wide variety of cell types either constitutively or after induction. They are involved in virtually every aspect of immunity and inflammation, including development and functioning of the immune system, cell proliferation and differentiation, cellular recruitment and activation, and regulation of cellular interactions with extracellular matrix proteins. Cytokines mediate the communication both locally between cells and tissues and distantly between organs [12,13]. They exert their functions in an autocrine, paracrine, or endocrine matter by binding to highly specific cell-surface receptors on target cells. Subsequently, intracellular signaling cascades are initiated that cause changes in gene expression. The cellular response is influenced by the differentiation status, the configuration and distribution of cytokine receptors, the mechanisms of cell attachment to the extracellular matrix, and the current status of cell signaling. Cytokines exhibit a few important characteristics, one of which is pleiotropy, which means that the same cytokine may have different activities on different cell types. Depending on the situation, different cytokines may have the same activity, a term called redundancy. Cytokines often act together and amplify the effects of one another. This synergy is often a prerequisite to express optimal functions. Numerous cytokines have both pro-inflammatory and anti-inflammatory potential [12,13]. Based on the fact that cytokines often affect the synthesis of other cytokines, inhibiting or assisting one cytokine pathway may influence other cytokine pathways, possibly leading to unpredictable implications [14].

Cytokines in the radiation response of skin

Although during recent years much progress has been made in dissecting the complex cytokine network, the role of cytokines in the pathophysiology of the cutaneous radiation reaction is only beginning to be elucidated. Cytokines are of utmost importance for signaling between cells and tissues and there is increasing evidence that they constitute a humoral component of the response of cells and tissues to radiation exposure [15,16]. Recently, a new concept has emerged regarding the induction of radiation damages. It suggests that a cascade of cytokines is initiated immediately after irradiation and during the clinically silent stage, persists for long periods of time, and leads to the development of late radiation damage [16].

Previous studies indicate that the major cytokines in the response of skin cells to ionizing radiation include interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β. In addition, skin cells have the potential to release granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemotactic cytokines such as IL-8 and eotaxin following ionizing radiation.

Interleukin-1

IL-1 is one of the few cytokines that has been demonstrated to be directly induced by ionizing radiation [5,17,18]. This pro-inflammatory cytokine exists in two forms, IL-1α and IL-1β, which have similar biological activities and both interact with the same receptor. IL-1 is of utmost importance in the acute-phase inflammatory response and the regulation of hematopoiesis. It is able to activate T lymphocytes, to enhance proliferation of B cells, and to stimulate adherence of leukocytes to the endothelium by upregulation of adhesion molecule expression. Some of the most relevant functions of IL-1 with respect to late effects of ionizing radiation exposure are the stimulation of proliferation of keratinocytes and fibroblasts and the induction of matrix metalloproteases (MMP) and collagen synthesis [19–22].

IL-1 is primarily produced by monocytes and macrophages but also by keratinocytes, fibroblasts, endothelial cells, and numerous other cells. The synthesis of IL-1 is induced by a variety of stimuli including endotoxin, other cytokines, and microbial and viral antigens, as well as ionizing radiation. In skin from C57BL/6 mice locally irradiated with a single dose of 30 Gy, keratinocytes have been identified as the major cell type responsible for production of IL-1. It has been demonstrated in murine skin that IL-1β protein levels increased immediately after ionizing radiation and remained chronically elevated, whereas IL-1α protein levels increased slowly with time after irradiation [17]. Parallel studies from this group on primary human keratinocytes revealed that ionizing radiation with 10 Gy caused an induction of IL-1 at both the mRNA and protein level [17]. A radiation-induced IL-1β mRNA expression has also been found in HaCaT cells, a spontaneously immortalized, nontumorigenic human keratinocyte cell line, as well as in the human epithelial HeLa cell line and in the human promyelocytic leukemia cell line HL60 following exposure to 40 Gy of ionizing radiation [5]. In a study by Koike et al.

[23], gene expression in normal human epidermal keratinocytes after ionizing radiation with 5 Gy was evaluated using microarray analysis. The data revealed that IL-1α mRNA levels were upregulated in irradiated keratinocytes as compared to unirradiated controls at 3 hours postirradiation [23].

Recently, data have been presented demonstrating a transient expression of IL-1 mRNA in Syrian hamster embryo fibroblasts within 3 hours after irradiation with low doses (0.75 to 0.9 Gy) [24]. In various in vitro and in vivo studies, IL-1β has been shown to be associated with increased expression levels of MMP [17,25,26]. MMP are a family of secreted proteolytic enzymes that have the capability to degrade constituents of the basal membrane and the extracellular matrix [27]. The biological activities of these proteases are subject to a complex regulatory system that includes specific tissue inhibitors of MMP (TIMP). The MMP/TIMP counterbalancing system plays a major role in several pathological conditions including skin fibrosis [28]. Based on above data, IL-1 signaling has been postulated to play a critical role in early dermatitis and radiation-induced skin fibrosis [17].

Interleukin-6

IL-6 is a pluripotent cytokine that is a major mediator of the acute inflammatory response. The various effects of IL-6 include differentiation of B lymphocytes, antibody formation, and activation of natural killer cells and cytotoxic T lymphocytes. IL-6 shares several biological functions with IL-1, including induction of pyrexia and production of acute-phase proteins [29–31]. In addition to these pro-inflammatory effects, IL-6 exerts several anti-inflammatory effects. Whereas both IL-1 and TNF-α induce synthesis of IL-6, IL-6 inhibits IL-1 and TNF-α synthesis [32]. The most important source of IL-6 is mononuclear phagocytic cells, but IL-6 is also produced by fibroblasts, keratinocytes, endothelial cells, T and B lymphocytes, hepatocytes, and bone marrow cells [30].

In previous studies, ionizing radiation has been shown to modulate the expression of IL-6 in different human cell types such as fibroblasts, keratinocytes, and epithelial cells [33–36]. Human embryonic lung fibroblasts FH109 have been reported to respond to a single dose of 5 Gy with an upregulation of IL-6 protein expression levels as early as 6 hours after irradiation [34]. The elevated IL-6 levels in the supernatant of irradiated fibroblasts were reflected by increased IL-6 mRNA expression levels [34]. It is well established that the IL-6 promoter contains a large number of DNA binding sites for inducible transcription factors including nuclear factor (NF)-κB, activator protein (AP)-1, AP-2, NF-IL-6, and cAMP-responsive element binding protein (CREB) [37–40]. The binding activity of three of them, namely NF-κB, AP-1, and CREB, has been demonstrated to be induced by ionizing radiation exposure [33,34,41,42]. In a study by Brach et al. [34], ionizing radiation of fibroblasts with 5 Gy has been reported to result in activation of both NF-κB and AP-1 with different time kinetics. Activation of the IL-6 promoter by ionizing radiation could not be detected after truncation of the NF-κB recognition sequence [34]. The authors have shown that both activation of transcription factors and induction of IL-6 mRNA expression occur independently of de novo protein synthesis, indicating that ionizing radiation causes posttranslational modifications of preexisting NF-κB and AP-1 proteins [34]. Increased IL-6 protein levels following ionizing radiation have also been observed in HaCaT and HeLa cells which was accompanied by accumulation of IL-6 transcripts [35,36]. In HeLa cells, IL-6 expression has been demonstrated to be dose-dependently increased up to a single dose of 20 Gy [36]. The radiation-induced upregulation of IL-6 expression could be inhibited in a dose-dependent manner by pretreatment of epithelial cells with corticosteroid derivatives hydrocortisone, dexamethasone, or mometasone furoate prior to ionizing radiation [36]. The IL-6 promoter contains at least four glucocorticoid-responsive elements (GRE), which bind complexes of corticosteroids with intracellular receptors and are located in positions of transcription factor binding motifs. It has been speculated that the bound corticosteroid receptor complexes inhibit the recognition of transcription factors involved in activation of the IL-6 promoter [43]. Thus, corticosteroids might be useful to suppress cytokine production as a consequence of cutaneous inflammation after ionizing radiation.

Tumor necrosis factor-α

TNF-α is a principal mediator of the cellular immune response with pleiotropic effects. In addition to mononuclear phagocytes, which represent its major source, TNF-α is secreted by neutrophils, lymphocytes, and natural killer cells following stimulation, but also by unstimulated cells, fibroblasts, endothelial cells, mast cells, and smooth-muscle cells [44]. The synthesis of TNF-α is induced by many different stimuli including bacterial lipopolysaccharides, interferons, and radiation. TNF-α is produced by processing of a precursor protein that is mediated by the TNF-α-converting enzyme [45]. The active form of TNF-α is a homotrimer. TNF-α exerts a variety of functions by binding to two distinct receptors on the cell surface, TNFR I (p55) and TNFR II (p75) [46]. It interacts with endothelial cells to induce adhesion molecule expression, thus facilitating the transendothelial migration of effector cells into inflamed tissues. TNF-α is also a potent activator of neutrophils; it mediates their cellular adherence, degranulation, chemotaxis, and oxidative burst. Just as IL-1, TNF-α stimulates fibroblast proliferation and induces synthesis of matrix metalloproteases [47,48].

In various studies, ionizing radiation has been demonstrated to induce TNF-α release by different cell types [5,6,49–52]. TNF-α protein expression has been shown by 5 of 13 human bone and soft-tissue sarcoma cell lines after ionizing radiation with 5 Gy [49]. Elevated TNF-α protein

expression levels in irradiated sarcoma cell lines were accompanied by increased levels of TNF-α mRNA [49]. Radiation-induced TNF-α mRNA expression has also been detected in HL60 cells [5,51], whereas no TNF-α induction occurred in normal human fibroblast cell lines and in four human epithelial cell lines irradiated with 5 Gy [49] and in HaCaT cells irradiated with 40 Gy [5]. However, in another study HaCaT cells were shown to express TNF-α protein when exposed to 5 Gy of ionizing radiation [6]. Recently, Beetz et al. [52] have reported that treatment of primary human keratinocytes with a single dose of 6 Gy results in TNF-α mRNA expression. It is well established that keratinocytes express TNF receptors on their cell surface [53]. As TNFR I expression on keratinocytes has been demonstrated to be increased after ionizing radiation exposure [52], it is tempting to speculate that synthesis of TNF-α by irradiated keratinocytes induces paracrine and autocrine mechanisms of cell activation and secondarily the production of cytokines. Both TNF-α and IL-1 have the capacity to induce IL-6 expression and all three cytokines are important mediators of inflammation, a key event in the cutaneous radiation reaction.

Transforming growth factor-β

TGF-β represents a family of peptides that exists in mammals in three distinct isoforms (TGF-β1, TGF-β2, and TGF-β3) displaying amino acid sequence homology between 70% and 80% [54]. Platelets are the major source of TGF-β, but the factor is synthesized also by several other cell types including macrophages, fibroblasts, keratinocytes, and endothelial cells. TGF-β1 is the prevalent form and is ubiquitously produced, while the other isoforms are expressed in a more limited spectrum of cells and tissues. TGF-β1 is released by the cells as a large latent complex including the TGF-β1 homodimer, the latency-associated peptide (LAP), and a second high-molecular-weight protein, the latent TGF-β binding protein (LTBP) [55,56]. LTBP is responsible for the interaction with extracellular matrix proteins. The latent TGF complex can be activated by various physico-chemical treatments or by proteases. TGF-β signals through transmembrane receptors with serine/threonine kinase activities that activate the recently discovered family of Smad proteins [57,58]. The Smad proteins are able to function as both transduction proteins and transcription factors. By interacting with its receptor, TGF-β induces activation and phosphorylation of different Smad proteins which then translocate to the nucleus to regulate the expression of various target genes. In previous studies, Smad 2 and Smad 3 have been shown to be involved in TGF-β signaling [59,60].

TGF-β1 is a multifunctional cytokine affecting many cellular processes including growth, differentiation, apoptosis, chemotaxis, and extracellular matrix production [54]. The biological activities of TGF-β1 vary according to the cell type studied and the tissue context involved [61]. TGF-β1 elicits a variety of rapid responses in cells including modulation of cell proliferation and apoptosis. TGF-β1 is a potent growth inhibitor for most cell types, mainly by inducing reversible G1 cell-cycle arrest and by effecting cyclin kinase activities and cell cycle–regulated genes. However, in contrast to its inhibitory effects on cell growth, TGF-β1 can promote proliferation of mesenchymal cells including fibroblasts and osteoblasts [62]. It can elicit apoptosis in a number of normal cells in primary culture such as human keratinocytes [63]. It has been postulated that the mechanisms underlying the TGF-β1-mediated cell death involve the generation of oxygen species.

TGF-β1 exerts immunosuppressive activities. It inhibits the proliferation of T and B lymphocytes, the cytotoxicity of natural killer cells, and the secretion of immunoglobulins by B lymphocytes, with the notable exception of IgA [54]. TGF-β1 also influences the cytotoxicity of mononuclear phagocytes by suppressing the production of NO [64]. It is a potent chemotactic factor for monocytes, neutrophils, mast cells, and fibroblasts [65–68]. In addition to mediating the recruitment of effector cells into inflamed tissues, TGF-β1 also activates neutrophils and induces macrophages to secrete cytokines, one of which is TGF-β1 itself. The TGF-β1 produced by macrophages can then stimulate matrix production by fibroblasts. TGF-β1 causes the deposition and remodeling of the extracellular matrix by simultaneously stimulating cells to increase the synthesis of most matrix proteins, such as collagen and fibronectin, to decrease synthesis of matrix-degrading proteases, and to enhance the production of tissue inhibitors of these proteases. TGF-β1 also modulates the expression of integrins [54]. Due to its diverse functions, TGF-β1 plays a central role in development and normal wound healing. It was proposed that deregulations of TGF-β1 functions are involved in the outcome of fibroproliferative disorders.

One of the most extensively characterized biological functions of TGF-β1 is its role in the pathogenesis of inflammatory and fibrotic diseases including radiation-induced skin fibrosis [69–72]. Early activation of TGF-β1 by ionizing radiation was analyzed in vivo in a well-characterized pig skin model [73]. Induction of both TGF-β1 mRNA and protein expression was detected in skin at 6 hours after γ irradiation with single doses between 16 and 64 Gy. Immunostaining on sections of irradiated pig skin revealed that TGF-β1 protein was secreted by fibroblasts, keratinocytes, and endothelial cells [73]. It is well established that the TGF-β1 promoter contains activator protein (AP-1) binding sites. In order to address the mechanisms underlying the early activation of TGF-β1 in irradiated skin, protein expression of Fos and Jun and their binding activity to the AP-1 consensus sequence after γ irradiation was evaluated. The data revealed that in parallel to TGF-β1 induction, Fos and Jun mRNA and protein were strongly induced by ionizing irradiation [73]. Analyzing nuclear proteins isolated from irradiated normal human

fibroblasts in gel shift assays, it has been demonstrated that binding to the consensus AP-1 sequence of the human TGF-β1 promoter was significantly increased within 6 hours after exposure to 16 Gy of γ irradiation [74]. These results suggest that stress-inducible TGF-β1 expression is mediated by the activation of AP-1 transcription factor [73,74]. In another study by the same authors, TGF-β1 gene expression was evaluated during the acute-subacute and the chronic phase after irradiation of pig skin with single doses in the range of 14 to 140 Gy [75]. TGF-β1 was increased 19-fold during the early erythematous phase, which started 3 weeks after irradiation. During the later phases of fibrosis, from 6 to 12 months after irradiation, TGF-β1 mRNA was highly expressed in the repaired skin and the underlying muscular fibrotic tissue, with 10-fold and eightfold increases, respectively. Immunoreactivity of TGF-β1 was found in myofibroblasts, collagen fibers, and capillary endothelial cells of repaired, fibrotic tissue [75]. Martin et al. [74] have reported strong induction of TGF-β1 by γ-rays both in skin and in an in vitro 3-D model of reconstituted skin. The authors have identified keratinocytes and fibroblasts as producers of elevated levels of TGF-β protein after irradiation [74]. Induction of TGF-β was also observed after β irradiation of mouse skin [76]. Localized irradiation of mice with a single dose of 50 Gy produced an acute moist desquamation which was resolved within 30 days and was followed by progressive remodeling of dermal tissues. The expression of TGF-β1 mRNA was monitored for 12 months after irradiation of mice and revealed three different waves of TGF-β1 expression, namely between 6 and 12 hours, between 14 and 28 days, and between 6 and 9 months after irradiation. The second peak correlated with the macroscopic reaction of the skin and the last peak with the presence of skin fibrosis [76]. Above data indicate the involvement of TGF-β1 both in the acute inflammatory phase and during tissue repair and the fibrotic response to ionizing radiation.

Previously, it has been shown that mice lacking Smad3, a key downstream mediator of TGF-β, exhibit accelerated healing of cutaneous injury induced by ionizing radiation, which is associated with reduced inflammation and accumulation of extracellular matrix proteins [77]. In a parallel study, it has been demonstrated that the acute tissue response to irradiation is markedly attenuated in Smad3 knockout mice and that incisional wounds made in skin 6 weeks after irradiation are more narrow and show an increased rate of epithelialization and reduced inflammatory cell infiltrates as compared to the wild-type control mice [78]. These data suggest that inhibition of Smad3 signaling might decrease tissue damage and reduce fibrosis after exposure to ionizing radiation [77,78].

Granulocyte-macrophage colony-stimulating factor

GM-CSF is one of the major hematopoietic growth factors belonging to a family of glycoproteins that stimulate proliferation and function of a wide variety of myeloid progenitor cells. It is mainly produced by T lymphocytes or nonhematopoietic cells [79]. Normal human fibroblasts also constitutively produce GM-CSF [80,81]. In a previous study, the effect of irradiation on GM-CSF expression in normal human embryonic lung fibroblasts WI38 has been investigated [82]. The data revealed that exposure of WI38 fibroblasts to doses between 20 and 80 Gy of γ-rays significantly increased GM-CSF expression both at the mRNA and protein level. The radiation-induced increase of GM-CSF expression occurred both dose- and time-dependently and was regulated by transcriptional and posttranscriptional mechanisms [82].

Interleukin-8

IL-8 differs from all other cytokines by its ability to mediate chemotaxis. It belongs to the large family of chemotactic cytokines, referred to as chemokines. IL-8 is primarily derived from mononuclear phagocytes, endothelial cells, and epithelial cells, but also from fibroblasts, keratinocytes, T lymphocytes, eosinophils, neutrophils, and melanocytes [83]. In many cell types, the synthesis of IL-8 is strongly stimulated by lipopolysaccharides, IL-1, and TNF-α. However, ionizing radiation, phytohemagglutinin, concanavalin A, double-stranded RNA, phorbol esters, and viruses may also function as inducers of IL-8 expression.

The production of IL-8 requires processing of a precursor protein by specific proteases, one of which is cathepsin L (IL-8 converting enzyme) [84]. The mature IL-8 protein is characterized by the presence of four conserved cysteine residues engaged in disulfide bridges. It is one of the most important chemoattractants for neutrophil granulocytes. In neutrophils, IL-8 causes a transient increase in cytosolic calcium levels. It stimulates neutrophil degranulation, the respiratory burst, and adherence to endothelial cells [85]. IL-8 binds to either CXCR1 or CXCR2, which are members of the G protein–coupled receptor protein family and expressed on many different cell types.

In human primary keratinocytes, ionizing radiation has been demonstrated to induce IL-8 expression [52]. The authors detected IL-8 transcripts as early as 3 hours after irradiation with a single dose of 6 Gy. The IL-8 mRNA expression slowly declined after 10 and 24 hours and returned to basal levels at 48 hours after irradiation [52]. The IL-8 promoter contains binding sites for the NF-κB and AP-1 transcription factors [86], both of which have been shown to be inducible by ionizing radiation [73,87]. It is tempting to speculate that radiation-induced activation of NF-κB and AP-1 represents a mechanism underlying the effect of ionizing radiation on IL-8 expression in keratinocytes. In normal diploid lung fibroblasts (HFL1) initially obtained from a human fetus, low doses of α particle (3.6–19 cGy) have been demonstrated to cause an increase in the production of IL-8 protein starting at 30 minutes after irradiation [88]. As revealed by northern blot analyses, the radiation-induced upregulation of IL-8 production in HFL1

cells was accompanied by increased IL-8 mRNA levels [50]. The increase of IL-8 protein secretion was inhibited by pretreatment of fibroblasts with 10^{-6} M dexamethasone prior to irradiation with 8.4 cGy [88]. Recently, a novel comprehensive gene expression analysis method has been performed on irradiated human diploid primary fibroblasts (HFLIII) derived from normal embryonic lung tissue [89]. In this study, the mRNA expression of more than 23,000 transcripts in HFLIII cells has been evaluated after irradiation of cells with low-dose ionizing radiation (1 cGy). The authors have found a significant upregulation of IL-8 mRNA expression in irradiated fibroblasts as early as 1 hour after irradiation [89]. Interestingly, the gene expression of three other chemokines of the CXC chemokine family, namely GRO-α (CXCL1), GRO-β (CXCL2), and GCP-2 (CXCL6), has also been detected to be increased more than twofold in irradiated HFLIII cells [89].

Eotaxin

Eotaxin is a potent eosinophil chemoattractant belonging to the CC chemokine family [90,91]. Eotaxin expression in humans was found in macrophages, mast cells, T cells, bronchial epithelial cells, dermal fibroblasts, and eosinophils [92–94]. In unstimulated human dermal fibroblasts, eotaxin mRNA is expressed at low levels and can be upregulated by stimulation with IL-1α and TNF-α within 6 hours [92,95]. Eotaxin induction by IL-1α is transient while long-term stimulation with TNF-α results in a further increase of eotaxin mRNA [92]. Unlike most other eosinophil chemoattractants, which are able to bind to several chemokine receptors, eotaxin only signals via one specific receptor, CCR3, prominently expressed on eosinophils [96]. Eosinophilia is a typical feature of the inflammatory reaction observed after therapeutic and accidental exposure to ionizing radiation. In a study by Huber et al. [97], the effect of ionizing radiation on eotaxin and CCR3 gene expression in human dermal fibroblasts has been evaluated. The results revealed that both eotaxin and CCR3 are induced in a time-dependent fashion after radiation exposure of fibroblasts to a single dose of 8 Gy. In addition, it has been observed that the radiation-induced increase in eotaxin and CCR3 expression occurs dose-dependently up to a single dose of 16 Gy [97]. The authors speculate that upregulation of CCR3 on dermal fibroblasts may lead to an autocrine feedback loop, resulting in an increased production of eosinophilic chemokines and thus in an elevated eosinophil recruitment. Interestingly, eotaxin expression was not induced by ionizing radiation in cultured primary keratinocytes [97]. Several consensus binding sites including sites for NF-IL6, AP-1, NF-κB, γ-interferon (IRF-1), and glucocorticoid signaling have been identified in the eotaxin promoter [95,98]. NF-κB and IRF-1 were found to be induced by ionizing radiation [87,97]. Thus, one may assume that the transcriptional activator NF-κB is involved in the radiation-induced modulation of eotaxin expression. The authors conclude from these data that the induction of signaling via eotaxin and CCR3 may be an important step leading to eosinophilia in radiotherapy patients [97].

In summary, ionizing radiation modulates the orchestrated communication between epidermal keratinocytes, dermal fibroblasts, and circulating and resident immunocompetent cells including dermal dendritic cells, neutrophils, eosinophils, and lymphocytes. Interactions between these different cell types within skin tissue are mediated in large part by cytokines. Synthesis and action of cytokines start immediately after radiation exposure and continue as a cascade during all stages of the cutaneous radiation syndrome leading to progressive late symptoms, the predominant of which is fibrosis.

The task of this review was to illustrate the standard of knowledge on cytokine modulation in skin by ionizing radiation. There are even much more and important data on the cutaneous cytokine network, which have not been investigated yet with respect to a possible modulation by ionizing radiation. The understanding of the pathophysiology of the radiation reaction moreover has moved from a predominantly single-organ approach up to the concept of multi-organ involvement and multi-organ failure [99]. In this context, particulary exploring the correlation between cytokine modulation in the bone marrow and corresponding events in other organs, such as the skin, remains a wide field of interest.

References

1. Peter RU. Klinische Aspekte des kutanen Strahlensyndroms nach Strahlenunfällen—Erfahrungen von Goiania und Tschernobyl. Aktuel Dermatol. 1993;19:364–367.
2. Peter RU. The cutaneous radiation syndrome. In: MacVittie TJ, Weiss JF, Browne D, eds. Advances in the Treatment of Radiation Injuries. Oxford, UK: Elsevier; 1996. p. 237–241.
3. Goldschmidt H. Dermatologische Röntgentherapie und Radiokarzinogenese. Hautarzt. 1982;33:183–190.
4. Hallahan DE. Radiation-mediated gene expression in the pathogenesis of the clinical radiation response. Semin Radiat Oncol. 1996;6:250–267.
5. Behrends U, Peter RU, Hintermeier-Knabe R, et al. Ionizing radiation induces human intercellular adhesion molecule-1 in vitro. J Invest Dermatol. 1994;103:726–730.
6. Müller K, Köhn FM, Port M, et al. Intercellular adhesion molecule-1: a consistent inflammatory marker of the cutaneous radiation reaction both in vitro and in vivo. Br J Dermatol. In press.
7. Heckmann M, Douwes K, Peter R, Degitz K. Vascular activation of adhesion molecule nmRNA and cell surface expression by ionizing radiation. Exp Cell Res. 1998;238:148–154.
8. Meineke V, Moede T, Gilbertz KP, et al. Protein kinase inhibitors modulate time-dependent effects of UV and ionizing radiation on ICAM-1 expression on human hepatoma cells. Int J Radiat Biol. 2002;78:577–583.
9. Hopewell JW. The skin: its structure and response to ionizing radiation. Int J Radiat Biol. 1990;57:751–773.
10. Bentzen SM, Thames HD, Overgaard M. Latent-time estimations for late cutaneous and subcutaneous radiation reactions in a single-follow-up clinical study. Radiother Oncol. 1989;15:267–274.

11. Lefaix JL, Daburon F. Diagnosis of acute localized irradiation lesions: A review of the French experimental experience. Health Phys. 1998; 75:375–384.
12. Nathan C, Sporn M. Cytokines in context. J Cell Biol. 1991;113:981–986.
13. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol. 2003;111(2 Suppl):S460–S475.
14. Schooltink H, Rose-John S. Cytokines as therapeutic drugs. J Interferon Cytokine Res. 2002;22:505–516.
15. Langberg CW, Hauer-Jensen M, Sung CC, Kane CJ. Expression of fibrogenic cytokines in rat small intestine after fractionated irradiation. Radiother Oncol. 1994;32:29–36.
16. Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN. A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys. 1995;33:99–109.
17. Liu W, Ding I, Chen K, et al. Interleukin 1β (IL1B) signaling is a critical component of radiation-induced skin fibrosis. Radiat Res. 2006; 165:181–191.
18. Hirota S, Tsujino K, Oshitani T, et al. Subcutaneous fibrosis after whole neck irradiation. Int J Radiat Oncol Biol Phys. 2002;52:937–943.
19. Romero LI, Zhang DN, Herron GS, Karasek MA. Interleukin-1 induces major phenotypic changes in human skin microvascular endothelial cells. J Cell Physiol. 1997;173:84–92.
20. Lambert CA, Lapiere CM, Nusgens BV. An interleukin-1 loop is induced in human skin fibroblasts upon stress relaxation in a three-dimensional collagen gel but is not involved in the up-regulation of matrix metalloproteinase 1. J Biol Chem. 1998;273:23143–23149.
21. Murphy JE, Robert C, Kupper TS. Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J Invest Dermatol. 2000;114:602–608.
22. Kessler-Becker D, Krieg T, Eckes B. Expression of pro-inflammatory markers by human dermal fibroblasts in a three-dimensional culture model is mediated by an autocrine interleukin-1 loop. Biochem J. 2004;379:351–358.
23. Koike M, Shiomi T, Koike A. Identification of skin injury–related genes induced by ionizing radiation in human keratinocytes using cDNA microarray. J Radiat Res. 2005;46:173–184.
24. Woloschak GE, Chang-Liu CM, Jones PS, Jones CA. Modulation of gene expression in Syrian hamster embryo cells following ionizing radiation. Cancer Res. 1990;50:339–344.
25. Yoo HG, Shin BA, Park JS, et al. IL-1β induces MMP-9 via reactive oxygen species and NF-κB in murine macrophage RAW 264.7 cells. Biochem Biophys Res Commun. 2002;298:251–256.
26. Brenneisen P, Wenk J, Wlaschek M, Krieg T, Scharffetter-Kochanek K. Activation of p70 ribosomal protein S6 kinase is an essential step in the DNA damage-dependent signaling pathway responsible for the ultraviolet B-mediated increase in interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) protein levels in human dermal fibroblasts. J Biol Chem. 2000;275:4336–4344.
27. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827–839.
28. Ulrich D, Noah EM, von Heimburg D, Pallua N. TIMP-1, MMP-2, MMP-9, and PIIINP as serum markers for skin fibrosis in patients following severe burn trauma. Plast Reconstr Surg. 2003;111:1423–1431.
29. Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB J. 1990;4:2860–2867.
30. Van Snick J. Interleukin-6: an overview. Annu Rev Immunol. 1990;8: 253–278.
31. Fattori E, Cappelletti M, Costa P, et al. Defective inflammatory response in interleukin 6–deficient mice. J Exp Med. 1994;180:1243–1250.
32. McIntosh JK, Jablons DM, Mule JJ, et al. In vivo induction of IL-6 by administration of exogenous cytokines and detection of de novo serum levels of IL-6 in tumor-bearing mice. J Immunol. 1989;143:162–167.
33. Tabata C, Kubo H, Tabata R, et al. All-trans retinoic acid modulates radiation-induced proliferation of lung fibroblasts via IL-6/IL-6R system. Am J Physiol Lung Cell Mol Physiol. 2006;290:L597–L606.
34. Brach MA, Gruss HJ, Kaisho T, Asano Y, Hirano T, Herrmann F. Ionizing radiation induces expression of interleukin 6 by human fibroblasts involving activation of nuclear factor-kappa B. J Biol Chem. 1993;268:8466–8472.
35. Petit-Frere C, Capulas E, Lyon DA, et al. Apoptosis and cytokine release induced by ionizing or ultraviolet B radiation in primary and immortalized human keratinocytes. Carcinogenesis. 2000;21:1087–1095.
36. Beetz A, Messer G, Oppel T, van Beuningen D, Peter RU, Kind P. Induction of interleukin 6 by ionizing radiation in a human epithelial cell line: control by corticosteroids. Int J Radiat Biol. 1997;72:33–43.
37. Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-κB transcription factor. Mol Cell Biol. 1990;10: 2327–2334.
38. Margulies L, Sehgal PB. Modulation of the human interleukin-6 promoter (IL-6) and transcription factor C/EBP β (NF-IL6) activity by p53 species. J Biol Chem. 1993;268:15096–15100.
39. Shimizu H, Mitomo K, Watanabe T, Okamoto S, Yamamoto K. Involvement of a NF-κB-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. Mol Cell Biol. 1990;10:561–568.
40. Zhang YH, Lin JX, Vilcek J. Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a κB-like sequence. Mol Cell Biol. 1990; 10:3818–3823.
41. Hallahan DE, Gius D, Kuchibhotla J, Sukhatme V, Kufe DW, Weichselbaum RR. Radiation signaling mediated by Jun activation following dissociation from a cell type–specific repressor. J Biol Chem. 1993; 268:4903–4907.
42. Sahijdak WM, Yang CR, Zuckerman JS, Meyers M, Boothman DA. Alterations in transcription factor binding in radioresistant human melanoma cells after ionizing radiation. Radiat Res. 1994;138(1 Suppl):S47–S51.
43. Ray A, LaForge KS, Sehgal PB. On the mechanism for efficient repression of the interleukin-6 promoter by glucocorticoids: enhancer, TATA box, and RNA start site (Inr motif) occlusion. Mol Cell Biol. 1990;10:5736–5746.
44. Beutler B, Cerami A. The biology of cachectin/TNF—a primary mediator of the host response. Annu Rev Immunol. 1989;7:625–655.
45. Perez C, Albert I, DeFay K, Zachariades N, Gooding L, Kriegler M. A nonsecretable cell surface mutant of tumor necrosis factor (TNF) kills by cell-to-cell contact. Cell. 1990;63:251–258.
46. Watanabe N, Neda H, Ohtusuka Y, et al. Signalling pathway of tumor necrosis factor in normal and tumor cells. Cancer Immunol Immunother. 1989;28:157–163.
47. Paulsson Y, Austgulen R, Hofsli E, Heldin CH, Westermark B, Nissen-Meyer J. Tumor necrosis factor–induced expression of platelet-derived growth factor A-chain messenger RNA in fibroblasts. Exp Cell Res. 1989;180:490–496.
48. Mauviel A. Cytokine regulation of metalloproteinase gene expression. J Cell Biochem. 1993;53:288–295.
49. Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor α mRNA after cellular exposure to ionizing radiation. Proc Natl Acad Sci U S A. 1989;86:10104–10107.
50. Sherman ML, Datta R, Hallahan DE, Weichselbaum RR, Kufe DW. Regulation of tumor necrosis factor gene expression by ionizing radiation in human myeloid leukemia cells and peripheral blood monocytes. J Clin Invest. 1991;87:1794–1797.

51. Hallahan DE, Virudachalam S, Sherman ML, Huberman E, Kufe DW, Weichselbaum RR. Tumor necrosis factor gene expression is mediated by protein kinase C following activation by ionizing radiation. Cancer Res. 1991;51:4565–4569.
52. Beetz A, Peter RU, Ried C, Ruzicka T, Michel G. Uniform induction of TNF-α and IL-8 in human keratinocytes by ionizing radiation is accompanied by non-uniform regulation of corresponding receptors. J Eur Acad Dermatol Venereol. 1996;7:188–190.
53. Trefzer U, Brockhaus M, Loetscher H, et al. 55-kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. J Invest Dermatol. 1991;97:911–916.
54. Roberts AB, Sporn MB. The transforming growth factor-β. In: Sporn MB, Roberts AB, eds. Handbook of Experimental Pharmacology. Peptide Growth Factors and their Receptors. New York: Springer-Verlag; 1990. p. 419–472.
55. Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB. Latent transforming growth factor-β: Structural features and mechanisms of activation. Kidney Int. 1997;51:1376–1382.
56. Pircher R, Jullien P, Lawrence DA. Transforming growth factor is stored in human platelets as a latent high molecular weight complex. Biochem Biophys Res Commun. 1986;136:30–37.
57. Derynck R, Feng XH. TGF-β receptor signaling. Biochem Biophys Acta. 1997;1333:105–150.
58. Massague J, Blain SW, Lo RS. TGF-β signaling in growth control, cancer, and heritable disorders. Cell. 2000;103:295–309.
59. Massague J. How cells read TGF-β signals. Nat Rev Mol Cell Biol. 2000;1:169–178.
60. Kretzschmar M, Massague J. Smads: Mediators and regulators of TGF-β signaling. Curr Opin Genet Dev. 1998;8:103–111.
61. Sporn MB. The importance of context in cytokine action. Kidney Int. 1997;51:1352–1354.
62. Battegay EJ, Raines EW, Seifert RA, Bowen-Pope DF, Ross R. TGF-β induces bimodal proliferation of connective tissue cells via complex control of an autocrine PDGF loop. Cell. 1990;63:515–524.
63. Benassi L, Ottani D, Fantini F, et al. 1,25-dihydroxyvitamin D3, TGF-β1, calcium, and UV B radiation induce apoptosis in cultured human keratinocytes. J Invest Dermatol. 1997;109:276–282.
64. Vodovotz Y, Bogdan C, Paik J, Xie QW, Nathan C. Mechanisms of suppression of macrophage nitric oxide release by TGF-β1. J Exp Med. 1993;178:605–613.
65. Wahl SM, Hunt DA, Wakefield LM, et al. Transforming growth factor type β induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci U S A. 1987;84:5788–5792.
66. Reibman J, Meixler S, Lee TC, et al. Transforming growth factor β1, a potent chemoattractant for human neutrophils, bypasses classic signal-transduction pathways. Proc Natl Acad Sci U S A. 1991;88:6805–6809.
67. Gruber BL, Marchese MJ, Kew RR. Transforming growth factor-β1 mediates mast cell chemotaxis. J Immunol. 1994;152:5860–5867.
68. Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor β. J Exp Med. 1987;165:251–256.
69. Barcellos-Hoff MH. How do tissues respond to damage at the cellular level? The role of cytokines in irradiated tissues. Radiat Res. 1998;150(5 Suppl):S109–S120.
70. Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. N Engl J Med. 1994;331:1286–1292.
71. Roberts AB, Sporn MB. Transforming growth factor-β. In: Clark RAF, ed. The molecular and cellular biology of wound repair. New York: Plenum Press; 1996. p. 275–308.
72. Branton MH, Kopp JB. TGF-β and fibrosis. Microbes Infect. 1999;1:1349–1365.
73. Martin M, Vozenin MC, Gault N, Crechet F, Pfarr CM, Lefaix JL. Co-activation of AP-1 activity and TGF-β1 gene expression in the stress response of normal skin cells to ionizing radiation. Oncogene. 1997;15:981–989.
74. Martin M, Lefaix J-L, Delanian S. TGF-β1 and radiation fibrosis: A master switch and a specific therapeutic target? Int J Radiat Oncol Biol Phys. 2000;47:277–290.
75. Martin M, Lefaix J-L, Pinton P, Crechet F, Daburon F. Temporal modulation of TGF-β1 and β-actin gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiat Res. 1993;134:63–70.
76. Randall K, Coggle JE. Long-term expression of transforming growth factor TGFβ1 in mouse skin after localized β-irradiation. Int J Radiat Biol. 1996;70:351–360.
77. Flanders KC, Sullivan CD, Fujii M, et al. Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. Am J Pathol. 2002;160:1057–1068.
78. Flanders KC, Major C, Arabshahi A, et al. Interference with transforming growth factor-β/Smad3 signaling results in accelerated healing of wounds in previously irradiated skin. Am J Pathol. 2003;163:2247–2257.
79. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. Blood. 1991;77:1131–1145.
80. Akashi M, Saito M, Koeffler HP. Lymphotoxin: stimulation and regulation of colony-stimulating factors in fibroblasts. Blood. 1989;74:2383–2390.
81. Akashi M, Loussararin AH, Adelman DC, Saito M, Koeffler HP. Role of lymphotoxin in expression of interleukin 6 in human fibroblasts. Stimulation and regulation. J Clin Invest. 1990;85:121–129.
82. Hachiya M, Suzuki G, Koeffler HP, Akashi M. Irradiation increases expression of GM-CSF in human fibroblasts by transcriptional and post-transcriptional regulation. Exp Cell Res. 1994;214:343–350.
83. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. Adv Immunol. 1994;55:97–179.
84. Ohashi K, Naruto M, Nakaki T, Sano E. Identification of interleukin-8 converting enzyme as cathepsin L. Biochim Biophys Acta. 2003;1649:30–39.
85. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest. 1989;84:1045–1049.
86. Yasumoto K, Okamoto S, Mukaida N, Murakami S, Mai M, Matsushima K. Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-κB-like binding sites of the interleukin 8 gene. J Biol Chem. 1992;267:22506–22511.
87. Brach MA, Hass R, Sherman ML, Gunji H, Weichselbaum R, Kufe D. Ionizing radiation induces expression and binding activity of the nuclear factor κB. J Clin Invest. 1991;88:691–695.
88. Narayanan PK, LaRue KE, Goodwin EH, Lehnert BE. Alpha particles induce the production of interleukin-8 by human cells. Radiat Res. 1999;152:57–63.
89. Fujimori A, Okayasu R, Ishihara H, et al. Extremely low dose ionizing radiation up-regulates CXC chemokines in normal human fibroblasts. Cancer Res. 2005;65:10159–10163.
90. Griffiths-Johnson DA, Collins PD, Rossi AG, Jose PJ, Williams TJ. The chemokine, eotaxin, activates guinea-pig eosinophils in vitro and causes their accumulation into the lung in vivo. Biochem Biophys Res Commun. 1993;197:1167–1172.
91. Jose PJ, Griffiths JD, Collins PD, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. J Exp Med. 1994;179:881–887.
92. Bartels J, Schlüter C, Richter E, et al. Human dermal fibroblasts express eotaxin: molecular cloning, mRNA expression, and identification of eotaxin sequence variants. Biochem Biophys Res Commun. 1996;225:1045–1051.
93. Lamkhioued B, Renzi PM, Abi-Younes S, et al. Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics

contributes to the chemotaxis of eosinophils to the site of inflammation. J Immunol. 1997;159:4593–4601.
94. Mattoli S, Stacey MA, Sun G, Bellini A, Martini M. Eotaxin expression and eosinophilic inflammation in asthma. Biochem Biophys Res Commun. 1997;236:299–301.
95. Hein H, Schlüter C, Kulke R, Christophers E, Schröder JM, Bartels J. Genomic organization, sequence, and transcriptional regulation of the human eotaxin gene. Biochem Biophys Res Commun. 1997;237:537–542.
96. Ying S, Robinson DS, Meng Q, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. Eur J Immunol. 1997;27:3507–3516.
97. Huber MA, Kraut N, Addicks T, Peter RU. Cell-type-dependent induction of eotaxin and CCR3 by ionizing radiation. Biochem Biophys Res Commun. 2000;269:546–552.
98. Garcia-Zepeda EA, Rothenberg ME, Weremowicz S, Sarafi MN, Morton CC, Luster AD. Genomic organization, complete sequence, and chromosomal location of the gene for human eotaxin (SCYA11), an eosinophil-specific CC chemokine. Genomics. 1997;41:471–476.
99. Meineke V, Fliedner TM. Radiation-induced multi-organ involvement and failure: challenges for radiation accident medical management and future research. BJR Suppl. 2005;27:196–200.