Death Receptors in Cutaneous Biology and Disease

Philippe Wehrli, Isabelle Viard, Roberto Bullani, Jürg Tschopp,* and Lars E. French

Department of Dermatology, Geneva University Medical School, Geneva, Switzerland; *Institute of Biochemistry, Lausanne University, Epalinges, Switzerland

Death receptors are a growing family of transmembrane proteins that can detect the presence of specific extracellular death signals and rapidly trigger cellular destruction by apoptosis. Expression and signaling by death receptors and their respective ligands is a tightly regulated process essential for key physiologic functions in a variety of organs, including the skin. Several death receptors and ligands, Fas and Fas ligand being the most important to date, are expressed in the skin and have proven to be essential in contributing to its functional integrity. Recent evidence has shown that Fas-induced keratinocyte apoptosis in response to ultraviolet light, prevents the accumulation of pro-carcinogenic p53 mutations by deleting ultraviolet-mutated keratinocytes. Furthermore, there is strong evidence that dysregulation of Fas expression and/or signaling contributes to the pathogenesis of toxic epidermal necrolysis, acute cutaneous graft *versus* host disease, contact hypersensitivity and melanoma metastasis. With these new developments, strategies for modulating the function of death receptor signaling pathways have emerged and provided novel therapeutic possibilities. Specific blockade of Fas, for example with intravenous immunoglobulin preparations that contain specific anti-Fas antibodies, has shown great promise in the treatment of toxic epidermal necrolysis and may also be useful in the treatment acute graft *versus* host disease. Likewise, induction of death signaling by ultraviolet light can lead to hapten-specific tolerance, and gene transfer of Fas ligand to dendritic cells can be used to induce antigen specific tolerance by deleting antigen-specific T cells. Further developments in this field may have important clinical implications in cutaneous disease. ***Key words: apoptosis/death receptors/Fas/skin. J Invest Dermatol 115:141–148, 2000***

Cell death by apoptosis is a tightly regulated physiologic process that enables the elimination of unwanted cells without causing an inflammatory response and its consequences. It is crucial for embryonic development and the maintenance of tissue homeostasis, but also for the defense against some infectious diseases and cancer. Many different stimuli such as cellular stress, growth factor withdrawal, DNA damage or signaling via selected cytokines can induce apoptosis. Although these stimuli trigger different upstream signaling pathways, most of them ultimately converge to an intracellular proteolytic cascade. This tightly regulated proteolysis leads to irreversible cleavage of selected proteins necessary for maintaining cell structure, as well as cleavage of proteins implicated in the control of the cell cycle and DNA synthesis and repair. Finally, these biochemical events lead to membrane blebbing, cellular shrinkage and condensation of chromatin, which are the characteristic morphologic features of apoptosis.

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Reprint requests to: Dr. Lars E. French, Department of Dermatology, Geneva University Medical School, 1211 Geneva 14, Switzerland. Email: french@cmu.unige.ch

Abbreviations: DcR, decoy receptor; DD, death domain; DED, death effector domain; DR, death receptor; FADD, Fas-associated death domain protein; FLICE, FADD-like ICE (caspase-8); IAP, inhibitor of apoptosis protein; RIP, receptor-interacting protein; TRADD, tumor necrosis factor receptor-associated death domain protein; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAMP, tumor necrosis factor-related-related apoptosis-mediating protein.

Cytokines of the tumor necrosis factor (TNF) family by binding to their specific cell-surface receptors, have the ability to regulate cellular proliferation and differentiation, but are also capable of inducing apoptosis. Certain cells are equipped with cell-surface sensors, named death receptors, that are able to detect the presence of specific extracellular death signals and rapidly trigger cellular destruction by apoptosis. During the last few years, several new death receptors and their ligands have been characterized (for review: Nagata, 1997; Ashkenazi and Dixit, 1998). Significant progress in our understanding of their mechanism of action and function has been made, some of which is pertinent to cutaneous biology and disease. In this review, we focus on the current knowledge of death receptor expression and function in the skin, including their implication in cutaneous diseases and promising therapeutic approaches to modulate their function.

Death receptors and intracellular signaling of apoptosis Death receptors are a subfamily of transmembrane proteins which belong to the TNF family of receptors. Six human death receptors [Fas (Apo-1, CD95), TNF-R1, TRAMP (WSL-1/Apo-3/DR-3/LARD), TRAIL (*T*NF *r*elated *a*poptosis *i*nducing *l*igand) -R1 (DR-4), TRAIL-R2 (DR-5, Apo-2, TRICK-2, KILLER), and DR-6], have been identified to date (Ashkenazi and Dixit, 1999, 1998). All are type I membrane proteins that contain two to four cysteine-rich extracellular domains and a cytoplasmic sequence named "death domain" (DD) (**Fig 1**). The latter couples each receptor to caspase cascades essential for the induction of apoptosis. The triggering of apoptosis following binding of a death ligand to its specific receptor is similar for all death receptor–ligand pairs. The best studied death-receptor signaling pathway to

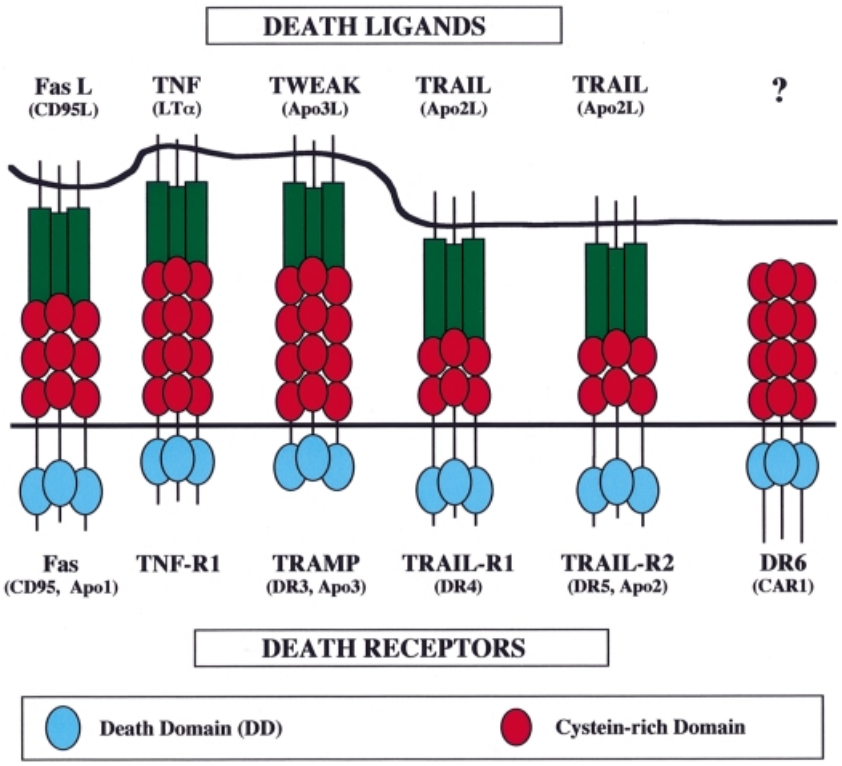

Figure 1. Death receptors and their ligands. Six human death receptors (Fas, TNF-R1, TRAMP, TRAIL-R1, TRAIL-R2, and DR-6), are known to date. All are type I membrane proteins that contain two to four cysteine-rich extracellular domains and a cytoplasmic sequence named ''death domain'' (DD). The known ligands for these death receptors are shown. DR6 is at present an orphan receptor.

date is that triggered by the binding of Fas ligand (FasL) to Fas. Schematically, multimerization or clustering of Fas upon binding of the membrane-bound form of FasL recruits the bipartite adaptor molecule FADD [Fas associated death-domain; composed of an amino terminal death effector domain (DED) and a carboxyl terminal (DD)]. FADD binds to Fas (via homophilic DD–DD interactions) and recruits the DED-containing caspase-8 (or probably also caspase-10) to the receptor via homophilic DED–DED interactions. Caspase-8 (or -10) within this newly formed death-inducing signaling complex then proteolytically activates itself and initiates apoptosis by subsequent cleavage of downstream effector caspases (caspases-3, -6, -7) (**Fig 2**). Depending on the cell type and the intensity of the death signal, recruitment of adaptor molecules can be detected within minutes, and apoptosis completed within a few hours. For Fas, there is an additional pathway that can signal apoptosis. It involves recruitment of Daxx, a protein that binds the cytosolic domain of Fas and links this receptor to an apoptosis pathway involving activation of Jun N-terminal kinase (JNK) (Yang *et al*, 1997). As several types of FADD-deficient cells show complete resistance to Fas-induced apoptosis, however, it appears that the Daxx pathway of coupling Fas to apoptosis is only functional in certain cell types (Yeh *et al*, 1998; Zhang *et al*, 1998).

Although all death receptors can transmit death signals, certain death receptors including TNF-R1 and TRAMP also have the ability to transmit signals that induce specific gene expression, such as NF-κB activation. This can result in a diverse set of cellular responses including proliferation, differentiation, and/or cellular activation, similarly to what classically occurs following signaling by TNF-R family members that do not have a death domain (CD40, CD30, CD27, etc.). In fact, signaling of apoptosis through TNF-R1 usually occurs only if protein synthesis is blocked. This suggests that TNF triggering of apoptosis is under most circumstances actively suppressed by cellular inhibitory proteins, thus privileging NF-κB or AP-1 mediated activation of proinflammatory and immunomodulatory genes.

The molecular control of death receptor signaling The expression of cell-surface molecules that can induce the death of adjacent cells is potentially dangerous to the organism as a whole. To avoid inappropriate cell death and disease, death receptor signals must therefore be tightly controlled. Over the past few years experimental evidence has shown that death-receptor apoptosis can be inhibited or controlled at several distinct points: at the receptor level (by receptor endocytosis, soluble ligands and/or decoy receptors), during signal transduction [FLICE inhibitory protein short (FLIPs)], and at the effector stage [caspase inhibitors: FLIP long (FLIPL), CrmA, p35, IAPs, etc.]. In several instances, such inhibitors have also been found to exist in infectious agents, to which they confer a survival advantage. This is the case, for example, for a new family of viral inhibitors of death receptor-mediated cell death named viral FLICE inhibitory protein (viral FLIP) (FLICE/caspase-8 inhibitory protein), that are found in several herpesviruses (including oncogenic human herpesvirus 8/ Kaposi's sarcoma associated herpesvirus and molluscipox virus) and inhibit DED–DED interactions between FADD and caspase-8 and -10 (Thome *et al*, 1997). Cellular homologs of viral FLIP were subsequently identified by us and others (cellular FLIP also named: Casper, iFLICE, FLAME-1, CASH, CLARP, MRIT, usurpin). They were shown to either structurally resemble caspase-8 except that they lack proteolytic activity (FLIPL), or contain only the two DED of caspase-8 (FLIPs) (Irmler *et al*, 1997; Tschopp *et al*, 1998). Their inhibition of caspase-8 activation by binding to FADD (in the case of FLIPs) or caspase-8 (in the case of FLIPL), renders cells resistant to apoptotic signals transmitted by Fas and all other death receptors tested to date (**Fig 2**).

Major physiologic functions of death receptors The majority of scientific work concerning the physiologic function of death receptors has been focused on the immune system. Death receptor pathways are crucial to the proper homeostasis and function of the immune system as exemplified by animal models and human pathologies related to Fas or FasL deficiencies. *Lpr* and *gld* mice present mutations that lead to absence of functional Fas and FasL, respectively. These mice suffer from a ''lymphoproliferative'' disease characterized by the accumulation of ($CD4^-$, $CD8^-$) lymphocytes, and an autoimmune disorder reminiscent of systemic lupus erythematosus in humans. This ''lymphoproliferative'' phenotype is due to ineffective peripheral deletion of autoreactive T cells by apoptosis. A similar phenotype called autoimmune lymphoproliferative syndrome that is due to Fas

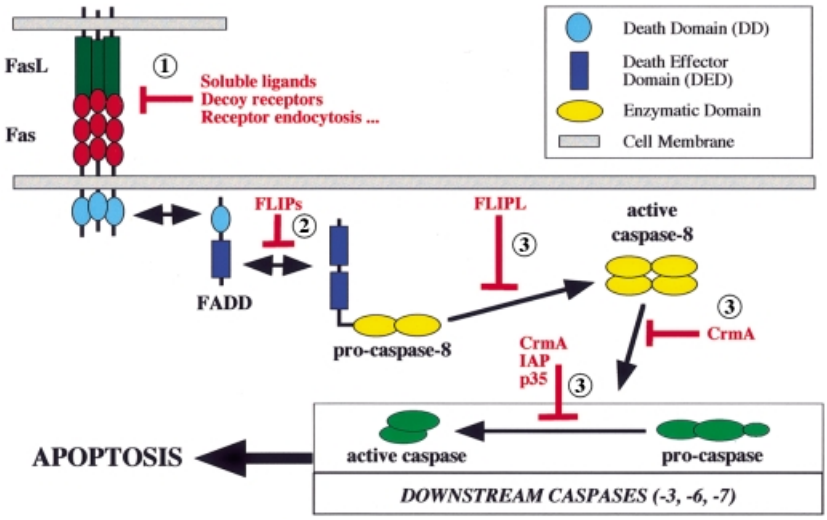

Figure 2. The Fas signaling pathway and its molecular control. Fas signaling is triggered on target cells by receptor tri(multi)merization induced upon contact with membrane bound FasL. Subsequent recruitment of FADD and pro-caspase-8 leads to upstream caspase (caspase-8) autoactivation that initiates apoptosis by subsequent cleavage of downstream effector caspases (caspases-3, -6, -7). Death receptor apoptosis can be inhibited at different points: receptor level (1), initiator stage (2), and effector stage (3).

mutations has now also been characterized in humans (Fisher *et al*, 1995; Rieux-Laucat *et al*, 1995). In addition to the above, Fas and FasL have also been shown to be required for: (i) downregulation of the immune responses through activation-induced cell death (Brunner *et al*, 1995; Dhein *et al*, 1995; Ju *et al*, 1995); (ii) cytotoxic T cell mediated lysis (Kägi *et al*, 1994; Lowin *et al*, 1994); and (iii) the killing of inflammatory cells that invade FasL expressing immune privileged tissues such as the testis and the eye (Bellgrau *et al*, 1995; Griffith *et al*, 1995). Little is yet known about the physiologic function(s) of the very recently identified death receptors TRAMP, TRAIL-R1, TRAIL-R2, and DR-6 or their ligands. TRAIL is constitutively expressed in many tissues; however, in peripheral blood T cells, natural killer cells and blood dendritic cells where it is not expressed in unstimulated state, TRAIL expression can be induced upon activation (Ashkenazi and Dixit, 1998; Thomas and Hersey, 1998; Zamai *et al*, 1998; Fanger *et al*, 1999; Kayagaki *et al*, 1999). Evidence suggests that TRAIL is implicated in activation induced T cell death, natural killer mediated cytolysis and CD4 cytotoxic lymphocyte mediated lysis.

Death receptor expression and function in keratinocytes The epidermis is a continually renewing tissue in which cell numbers are tightly regulated by an intricate balance between proliferation, differentiation and cell death. Morphologic and biochemical analyses have established that keratinocyte apoptosis does occur within the normal epidermis and during hair follicle regression, although it is still controversial whether keratinocyte terminal differentiation is a form of apoptosis or not (Budtz and Spies, 1989; McCall and Cohen, 1991; Gavrieli *et al*, 1992; Mitra *et al*, 1997; Gandarillas *et al*, 1999; Weil *et al*, 1999). TNF-R1, Fas, and TRAIL receptors are expressed by keratinocytes, but there is presently no evidence that they are functionally associated with keratinocyte cell death occurring during terminal differentiation. The expression and function of the Fas death receptor–ligand pair is the best studied of death receptor–ligands in the epidermis to date. By immunohistochemistry Fas has been located at the membrane of basal and suprabasal keratinocytes in normal human epidermis (Leithauser *et al*, 1993; Oishi *et al*, 1994; Sayama *et al*, 1994; Matsue *et al*, 1995). *In vitro*, primary keratinocytes also express Fas, and this death receptor is functional under basal conditions as triggering with FasL or agonistic anti-Fas antibodies can induce keratinocyte apoptosis (Sayama *et al*, 1994; Leverkus *et al*, 1997; Viard *et al*, 1998). Somewhat surprisingly, FasL was also recently shown to be expressed in the epidermis, where its expression is restricted to the basal layer of keratinocytes (Viard *et al*, 1998). Such coexpression of Fas and its ligand in adjacent cells of the same tissue would be expected to lead to apoptosis. This is not the case in the epidermis, however. Despite abundant Fas and FasL coexpression, keratinocyte apoptosis is quite a rare event in the epidermis under physiologic conditions, and exposure of Fas-sensitive target cells to cryostat sections of skin that express FasL does not result in target cell apoptosis (Viard *et al*, 1998). The reason why keratinocyte FasL is nonfunctional within the normal epidermis remains unclear at present, but may be due to its cellular localization (stocked intracellularly as opposed to expressed on the cell surface), or other control mechanisms known to regulate the lytic potential of FasL, such as metalloproteinase-mediated surface cleavage and inactivation (see **Fig 2**). On the other hand, however, subconfluent keratinocytes in culture not only express FasL, but have been shown by certain authors to induce apoptosis in Fas-positive cells (Berthou *et al*, 1997; Gutierrez-Steil *et al*, 1998; Arnold *et al*, 1999). Why keratinocyte FasL is lytic *in vitro*, but not *in vivo*, also remains to be determined. The expression of Fas and FasL by keratinocytes is subject to regulation. Basal expression can be significantly upregulated by different stimuli, including ultraviolet (UV), certain cytokines (interleukin-1β, TNF-α, interferon-γ, and interleukin-15) (Leverkus *et al*, 1997; Gutierrez-Steil *et al*, 1998; Arnold *et al*, 1999), and anti-cancer drugs (doxorubicin, methotrexate, cytarabine, etoposide, and cisplatin) (Moers *et al*, 1999). Upregulation of Fas by such stimuli enhances keratinocyte sensitivity to FasL, and increased FasL expression can confer keratinocytes with lytic potential *in vitro*.

Despite considerable knowledge of the expression and signaling potential of the Fas death receptor–ligand pair in keratinocytes *in vitro* and *in vivo*, its physiologic function in the epidermis has remained unknown. Very recent work has shed light on a key physiologic function of Fas and its ligand, namely protection against the mutagenic effects of UV light. First, Aragane *et al* (1998) showed that UV can induce keratinocyte apoptosis via direct aggregation and activation of Fas independently of FasL, and in the presence or absence of functional p53. Second, Hill *et al* (1999a) have shown that mice with mutant nonfunctional FasL are significantly more resistant to keratinocyte apoptosis (''sunburn cells'') as a consequence of UVB exposure, and furthermore accumulate UV-induced p53 mutations significantly more rapidly than control mice. Taken together, it appears therefore that: (i) UV can induce keratinocyte apoptosis by Fas–FasL interaction or by direct FasL-independent triggering of Fas; (ii) Fas can mediate apoptosis in response to UV even in the absence of functional p53; and (iii) Fas-mediated keratinocyte apoptosis in response to UV prevents the accumulation of pro-carcinogenic p53 mutations by deleting mutated keratinocytes through apoptosis. This experimental data strongly supports a physiologic role for the Fas–FasL system in the protection of the epidermis from the carcinogenic effects of UV.

Recently, a new death ligand named TRAIL and four receptors for which it is specific (TRAIL-R1, TRAIL-R2, TRAIL-R3, and TRAIL-R4) have been discovered. TRAIL-R1 and -R2 induce

Table I. Death receptors in cutaneous disease

Disease	Causal molecule (species)	Mechanism	References
Toxic epidermal necrolysis (TEN)	FasL (human)	Increased keratinocyte FasL expression and function	Viard *et al*, 1998
Acute graft *vs*. host disease (acute GVHD)	FasL (mouse)	Induction of apoptosis in skin, gut, and liver by donor lymphocyte FasL and perforin-granzyme	Baker *et al*, 1996 Brown *et al*, 1996 Tsukada *et al*, 1999
Basal cell carcinoma (BCC)	Fas + FasL (human)	Loss of Fas expression and upregulation of FasL: probably contributes to immune evasion	Buechner *et al*, 1997 Gutierrez-Steil *et al*, 1998
Melanoma	Fas + FasL (human)	Loss of Fas expression, Fas mutation: probably contributes to immune evasion	Hahne *et al*, 1996 Thomas and Hersey, 1998 Ugurel *et al*, 1999
Contact hypersensitivity (CHS)	FasL (mouse)	FasL contributes with perforin-granzyme to hapten-specific $CD8^+$ T cell cytotoxicity and CHS reaction	Kehren *et al*, 1999

Table II. Death receptors and ligands as targets or tools for therapy of cutaneous disease

Disease	Molecule used as target or tool	Therapeutic possibilities (examples)	References
Toxic epidermal necrolysis (TEN)	Fas (target)	Intravenous immunoglobulins that contain anti-Fas blocking antibodies	Viard *et al*, 1998
		Monoclonal anti-Fas blocking antibodies	–[a]
Acute graft *vs*. host disease (acute GVHD)	Fas + FasL (target)	Same as above	–[a] Hattori *et al*, 1998 Miwa *et al*, 1999
Basal cell carcinoma (BCC)	Fas (target)	Interferon-α (intralesional) to re-induce Fas expression and promote tumor involution by Fas-FasL mediated fratricide	Buechener *et al*, 1997
Psoriasis Atopic dermatitis (AD)	Fas + FasL (targets)	UV light: induces Fas-mediated apoptosis of skin infiltrating lymphocytes amongst other effects	Krueger *et al*, 1995 Ozawa *et al*, 1999 Morita *et al*, 1997
Cutaneous allergic diseases	FasL (tool)	FasL engineered dendritic cells (''killer'' DCs) pulsed with causal antigen: induces antigen-specific tolerance by killing antigen-specific T cells	Matsue *et al*, 1999
Tumors	TRAIL (tool)	Recombinant soluble TRAIL to selectively kill TRAIL sensitive tumor cells (e.g., melanoma)	Griffith and Lynch, 1998

[a]Hypothetical and not published.

apoptosis upon contact with TRAIL, whereas TRAIL-R3 and -R4, also known as decoy receptors, were shown to be competitive membrane-bound inhibitors of TRAIL signaling, as they lack the cytoplasmic intracellular death domain that is required for signaling death. TRAIL and all four TRAIL receptors have been shown to be expressed by normal keratinocytes (Kothny-Wilkes *et al*, 1998). Although normal keratinocytes express TRAIL receptors, they are resistant to apoptosis induced by recombinant TRAIL. Kothny-Wilkes *et al* (1998) have suggested that this is due to concomitant expression of the two decoy receptors (TRAIL-R3 and TRAIL-R4), as their expression is decreased in the transformed keratinocyte cell line HaCat, and this correlated with susceptibility to TRAIL-mediated apoptosis *in vitro*. Enhanced susceptibility of tumor cell types over their normal counterparts to TRAIL has been previously reported, and this could be of potential therapeutic interest for cancer therapy (for review French and Tschopp, 1999).

Death receptors in melanocytes and Langerhans cells Only few studies have addressed the issue of death receptor and ligand expression in melanocytes and Langerhans cells. TNF has been shown to be implicated in Langerhans cell functions such as migration and maturation, but not in TNF-R1 mediated cell death, most likely because these cells do not readily express TNF-R1 (Larregina *et al*, 1996). Immunohistochemical studies suggest that Langerhans cells express Fas (Oishi *et al*, 1994), and clearance of the antigen-bearing Langerhans cells appears to be dependent on Fas-mediated cell death, as it is significantly delayed in Fas- and FasL-deficient mice 2 d after skin painting with fluorescein isothiocyanate (Kawamura *et al*, 1999). Although not reported, primary melanocytes as assessed by RNase protection analysis do contain mRNA for TNF-R1 and Fas, but not their respective ligands (Bullani, Wehrli and French, unpublished results). Fas can also be detected by FACS analysis on the surface of melanocytes from primary cultures, and this correlates with their ability to signal death upon exposure to recombinant FasL, although signaling appears less effective than in cells of the lymphocytic lineage (Bullani, Wehrli, and French, unpublished results). Finally, the four TRAIL receptors are detectable in normal melanocytes at the RNA level, but not at the protein level, and consequently melanocytes have been shown to be resistant to cell death induced by recombinant TRAIL (Zhang *et al*, 1999).

Death receptor signaling and cutaneous disease To date, our knowledge of the implication of death receptor pathways in the pathogenesis of skin disease is dominated by that of Fas. Diseases that affect the skin, and for which evidence of death receptor involvement exists include toxic epidermal necrolysis (TEN), acute graft *versus* host disease (GVHD), nonmelanoma skin cancer, melanoma, and contact hypersensitivity (CHS; **Table I**).

Toxic epidermal necrolysis TEN (Lyell's syndrome) is a severe drug reaction in which the abrupt onset of massive keratinocyte apoptosis results in the detachment of large sheets of epidermis from the underlying dermis (Paul *et al*, 1996). We have recently shown that this process is associated with highly increased keratinocyte FasL expression, together with conserved levels of keratinocyte Fas expression *in vivo* (Viard *et al*, 1998). Functional experiments performed by overlaying cryostat sections of lesional skin with Fas-sensitive cells as targets, has demonstrated that keratinocyte FasL is cytolytically active in TEN. This cytolytic activity could be blocked with monoclonal antibodies that interfere with the interaction of Fas and FasL, thus supporting the hypothesis that increased keratinocyte FasL expression is responsible for the keratinocyte apoptosis that characterizes TEN. It also provided a rationale for molecular strategies to treat TEN.

Acute cutaneous GVHD GVHD is a frequent complication of allogeneic bone marrow transplantation that results from the attack of transplanted donor lymphocytes against the hosts skin, liver, and gut. Although in its aggressive form, acute GVHD can be a serious life-threatening disease, in its mild form, the disease can be useful due to its anti-tumor effect. Increased apoptosis within target tissues is a key element of the pathogenesis of the immune-mediated skin, gut and liver damage in acute GVHD (Gilliam *et al*, 1996; Sale, 1996). Using cytotoxic T lymphocytes from mice deficient in both functional FasL and perforin (Kägi *et al*, 1994; Lowin *et al*, 1994), it has been shown that development of lethal acute GVHD in appropriate transplantation models can be virtually completely prevented (Braun *et al*, 1996; Baker *et al*, 1996). Delayed mortality was also observed in animals grafted with cells deficient in only one of the major cytolytic pathways (perforin or FasL). Recipients grafted with allogeneic FasL-defective donor T cells only exhibited minimal signs of hepatic and cutaneous GVHD pathology as compared with controls (Baker *et al*, 1996). Whether inhibition of acute GVHD by blockade of cytolytic pathways is feasible without compromising a graft *versus* leukemia effect has only recently been assessed. Tsukada *et al* (1999) have been able to demonstrate that selective inactivation of Fas, perforin or TNF all significantly inhibit murine acute GVHD, but only blockade of Fas does so without also inhibiting the graft *versus* leukemia reaction. Similarly, inhibition of FasL with antibodies has been shown to have a therapeutic effect on murine acute GVHD (Hattori *et al*, 1998; Miwa *et al*, 1999). Taken together, these studies of acute GVHD show that lymphocyte FasL, which is induced during acute GVHD, is involved in the pathogenesis of tissue damage by inducing Fas-mediated apoptosis in target organs (skin, gut, and liver), and thereby contributes to the mortality of GVHD.

Nonmelanoma skin cancer Basal cell carcinomas are the most common type of skin cancer in humans. It has been shown that in contrast to normal basal keratinocytes, basal cell carcinoma cells have low to undetectable levels of Fas expression, whereas their FasL expression is quite strong (Buechner *et al*, 1997; Gutierrez-Steil *et al*, 1998). The molecular mechanism of loss of Fas expression in these cells is unknown but could be an indirect consequence of the oncogenic process. As an example, accumulating experimental evidence in fibroblastic, epidermal and melanocytic cell lines shows that oncogenic Ras (H-Ras) downregulates Fas surface expression as a consequence of promoter methylation. This loss of Fas expression correlates with decreased sensitivity to Fas-mediated cell death, thus contributing to tumorigenesis as shown in mouse models (Fenton *et al*, 1998; Peli *et al*, 1999). In basal cell carcinomas, loss of tumor cell Fas expression probably contributes to the pathogenesis at some stage, as reinduction of Fas expression by intralesional interferon-α injection causes rapid tumor regression as a result of tumor cell apoptosis (Buechner *et al*, 1997). Chronic UV exposure and p53 mutations have been shown to contribute to the pathogenesis of squamous cell carcinomas (Ziegler *et al*, 1994; Brash *et al*, 1996). As reported above, the Fas system is implicated in UV-induced keratinocyte apoptosis, and prevents the accumulation of p53 mutations (Hill *et al*, 1999a). Although not directly proven to date, it is likely that the disruption of the Fas signaling process may contribute to squamous cell carcinoma development by favoring the accumulation of keratinocyte p53 mutations that are central to the pathogenesis of squamous cell carcinomas.

Melanoma Downregulation/loss of Fas expression has been found in a variety of malignancies (e.g., pulmonary adenocarcinomas, esophageal cancer), including melanoma (Leithauser *et al*, 1993; Hahne *et al*, 1996; Shin *et al*, 1999; Sprecher *et al*, 1999), and as a result, melanomas acquire resistance to FasL, as established by cell death assays performed *in vitro* (Thomas and Hersey, 1998; Ugurel *et al*, 1999). Recent work in a mouse model has demonstrated that Fas downregulation could favor the metastatic behavior of melanoma (Owen-Schaub *et al*, 1998), thus showing that Fas resistance confers a spreading advantage to the tumor; however, the cause of loss of Fas expression has not yet been identified. Again, Ras mutations may be implicated. Considerable work supports the role of N-ras and H-ras mutations in promoting melanoma (Ball *et al*, 1994; Jafari *et al*, 1995; Wagner *et al*, 1995). Furthermore, recently a mouse model of spontaneously occurring melanoma has been developed, in which melanocyte-specific expression of activated H-Ras is regulated in mice null for INK4a. Using this model, melanoma genesis and maintenance has been shown to be strictly dependent on the expression of the oncogene H-ras (Chin *et al*, 1999). As activated H-Ras can cause resistance to FasL-mediated apoptosis (Fenton *et al*, 1998; Peli *et al*, 1999), it is interesting to speculate that Fas downregulation may be as a consequence of this, contributing to the pathogenesis of melanoma.

In addition to the demonstrated loss of Fas surface expression, mutations of the death domain of Fas have recently been described in a small percentage of melanomas (Shin *et al*, 1999). Furthermore, abundant expression of a cellular inhibitor of death receptor signaling named cellular FLIP has been shown to contribute to melanoma resistance to FasL in some cases (Irmler *et al*, 1997; Griffith *et al*, 1998; Zhang *et al*, 1999). The physiologic relevance of enhanced cellular FLIP expression by tumor cells has recently been established; it clearly favors escape from T cell immunity despite a functional cytotoxic T lymphocyte perforin–granzyme cytolytic pathway (Djerbi *et al*, 1999; Medema *et al*, 1999).

In vivo various types of nonhematopoietic tumors strongly express FasL, and there is considerable evidence that tumor FasL favors immune escape by causing apoptosis of Fas-positive tumor infiltrating immune effector cells (O'Connell *et al*, 1999). In melanoma, FasL expression has been shown to be stronger in metastatic as compared with primary melanomas (Ekmekcioglu *et al*, 1999; Terheyden *et al*, 1999) and normal melanocytes (Hahne *et al*, 1996). Considerable controversy has, however, arisen with respect to the functional relevance of FasL expression in melanoma. First, there is prevailing evidence that FasL is not expressed at the protein level and/or functional in melanoma cell lines (Arai *et al*, 1997; Chappell *et al*, 1999; Zaks *et al*, 1999), and second, *in vivo* experiments of tumor growth using FasL-transfected tumor have shown, against all expectations, enhanced rejection of these tumor cells due to enhanced neutrophil infiltration (Kang *et al*, 1997; Chen *et al*, 1998; Drozdzik *et al*, 1998). Presently, the *in vivo* consequences of FasL expression in melanoma are thus still unclear.

As previously stated, tumor cell lines are more sensitive to TRAIL than their normal counterparts. This holds true for melanoma too, in which TRAIL but not other members of the TNF family, induce apoptosis in approximately two-thirds of melanoma cell lines (Griffith *et al*, 1998; Thomas and Hersey, 1998). Promising experiments have been performed in mice, showing that TRAIL administration is effective in reducing/preventing tumor growth in mammary and colon adenocarcinoma tumor models (Ashkenazi and Dixit, 1999; Walczak *et al*, 1999). Interestingly, TRAIL administration was devoid of detectable toxicity. It may thus hold promise for the treatment of these types of tumors, and possibly also melanoma in humans.

Contact hypersensitivity CHS is an inflammatory skin disease that is mediated by T cells following contact of the skin of sensitized individuals with contact allergens. Evidence suggests that $CD8^+$ T cells are implicated in CHS, and recently the implication of $CD8^+$ T cell FasL and perforin in CHS has been evaluated in mice. Using mice deficient in perforin and/or FasL, it has been shown that double deficient mice (perforin −/−, FasL deficient) were able to develop hapten-specific $CD8^+$ T cells but not a CHS reaction. This is because these mice did not develop hapten-specific cytotoxic $CD8^+$ T cells, thus demonstrating that the CHS reaction is dependent on cytotoxic activity. In the disease model studied, FasL and perforin could complement for each other. Thus mice deficient in only FasL or perforin developed both hapten-specific cytotoxic $CD8^+$ T cells and CHS (Kehren *et al*, 1999).

Potential for therapeutic modulation of death receptor signaling in cutaneous disease As reviewed above, death receptor pathways, although only recently discovered, have already been implicated in the pathogenesis of several disease conditions. Developing strategies to inhibit or redirect these molecules for therapeutic purposes has therefore rapidly become a realistic and exciting challenge. Interesting examples of such strategies and their potential include: inhibition of Fas signaling with intravenous immunoglobulins, induction of Fas-mediated cell death and tolerance following UV exposure, induction of antigen-specific tolerance via killer dendritic cells, and selective induction of tumor cell death with TRAIL (**Table II**).

Inhibition of Fas signaling with intravenous immunoglobulins The demonstration that Fas-mediated cell death induced *in vitro* by lesional skin of patients with TEN can be abrogated by blockade with monoclonal antibodies against FasL or by Fas:Fc, suggests that blocking antibodies may be useful in the treatment of TEN. We have recently shown that commercial preparations of intravenous immunoglobulins contain antibodies against Fas that are able to block the binding of FasL to Fas (Viard *et al*, 1998). Furthermore intravenous immunoglobulins, by blocking Fas, potently inhibit cell death mediated by recombinant FasL *in vitro*. When used in high doses (0.75 g per kg per day for 4 consecutive days) to treat patients with TEN, intravenous immunoglobulins consistently and rapidly blocked the progression of skin detachment and disease in 17 of the 18 patients treated to date (Viard *et al*, 1998, and unpublished results). Although it is our impression that late treatment onset, low-dose treatment and the coexistence of underlying debilitating disease may lead to nonresponse, a controlled trial is required to establish the efficacy of intravenous immunoglobulins, and the best treatment modality in TEN.

Induction of Fas-mediated cell death and tolerance following UV exposure UV light is well known to the dermatologist for its immunosuppressive properties. This is the rationale for its use in the treatment of diseases such as atopic dermatitis and psoriasis. Upon exposure of the skin to UV light, hapten-specific tolerance can be induced as a consequence of the development of hapten specific T suppressor cells. The groups of Schwarz and Hill have independently shown, using mutant mice, that Fas and FasL are essential for the development of UV-induced tolerance (Schwarz *et al*, 1998; Hill *et al*, 1999b). Although the exact mechanism by which the Fas system mediates tolerance to contact sensitizers following UV exposure remains unclear, it appears that this implicates dendritic cell (DC) death mediated by FasL, the latter possibly resulting from yet undefined signaling events between UV-induced T suppressor cells and the concerned DC. UV mediates therapeutic responses in inflammatory skin diseases also by inducing epidermal and dermal lymphocyte apoptosis (Krueger *et al*, 1995; Ozawa *et al*, 1999). Recently, Morita *et al* (1997) have demonstrated that UV-mediated lymphocyte death is induced via Fas signaling. Indeed, in patients with atopic dermatitis, Fas-mediated apoptosis of skin infiltrating T helper cells has been shown to contribute to cutaneous T cell depletion.

Induction of antigen-specific tolerance via killer dendritic cells The ability to specifically suppress immune responses would be of great value for the treatment of cutaneous and systemic diseases including allergic and autoimmune diseases. Recently, the group of Takashima has been able to induce antigen-specific tolerance in mice successfully, by generating DC that constitutively express FasL as a result of gene transfer (Matsue *et al*, 1999). These FasL expressing DC were shown to deliver death instead of activation signals to T cells after antigen-specific interaction *in vitro*, and were therefore named "killer DC". When tested *in vivo* by injection prior to antigenic challenge, these killer DC could induce significant antigen-specific immunosuppression (Matsue *et al*, 1999). Moreover, when injected in a "therapeutic" manner after sensitization, "killer DC" could also significantly suppress the immune response (Matsue *et al*, 1999). If transposable to the human setting, and devoid of unforeseen side-effects, such an approach may prove to be a very useful therapeutic tool.

Selective induction of tumor cell death with TRAIL Based on work demonstrating that a high proportion of cancer cell lines are sensitive to the cytotoxic effects of TRAIL *in vitro*, whereas most normal (nontransformed) cells are resistant (for review Griffith and Lynch, 1998), the *in vivo* tolerance and anti-tumor activity of a genetically engineered soluble form of TRAIL was recently tested in mice. Systemic administration of high doses of recombinant soluble TRAIL has been shown to be devoid of detectable side-effects in mice and effective in selectively targeting tumors. When repetitively injected intravenously or intraperitoneally, TRAIL can as a single agent, induce shrinkage, and in some cases, complete eradication of subcutaneously implanted mammary and colon carcinomas. This tumoricidal effect is due to selective activation of tumor cell death, presumably as a result of TRAIL binding to TRAIL-R1 and/or TRAIL-R2. Given that approximately 50% of the human cell lines tested to date, including certain cell lines derived from melanomas, lymphomas, colon, lung, and breast carcinomas, are sensitive to TRAIL-induced apoptosis (for review Griffith and Lynch, 1998), this molecule may in the future prove useful in the clinical setting, alone or in combination with other cancer treatments.

CONCLUSIONS AND PERSPECTIVES

Since the discovery of the first prototypic death receptor Fas in 1989, substantial progress has been made in our understanding of the mechanism of action and function of death receptors. A total of six death receptors have now been identified and their downstream signaling pathways leading to cell death have been characterized. Loss of function mutations in the Fas system have highlighted the importance of death receptor signaling in the maintenance of tissue homeostasis, specifically within the immune system. As outlined in this review, death receptors also contribute their share to cutaneous biology as illustrated by the role of Fas in protecting the epidermis from the mutagenic effects of UV light. Death receptors are double-edged swords, however. On the one hand they protect tissues, and on the other, improper regulation of their function results in rapid tissue destruction that can be life-threatening, as illustrated by TEN.

In the future, research will provide us with further insight into biologic roles of death receptors and their ligands. Identification of new defects in death receptor-mediated apoptosis will also contribute to our understanding of skin disease. Furthermore, given the promising results obtained to date, efforts will certainly be concentrated on defining how modulation of death receptor pathways can be applied for the treatment of human diseases including those of the skin. If properly targeted, such therapies are likely to prove useful for inhibiting death receptor-mediated tissue destructive processes, selectively killing tumor cells, and suppressing unwanted immune responses. Undoubtedly, past and future discoveries in this field will set the basis for the development of novel therapeutic approaches for systemic and cutaneous disease.

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