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Graft-versus-Host Disease of the Skin: Life and Death on the Epidermal Edge

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ABSTRACT

Despite impressive advances in the field of allogeneic hematopoietic transplantation, graft versus host disease (GVHD) remains a significant obstacle to be overcome; it would enhance the safety and efficacy of this life-saving therapy. This review provides a framework for understanding the molecular and cellular basis underlying GVHD. We propose a 3-phase model of GVHD that highlights the importance of the conditioning regimen on the recipient tissues administered prior to infusion of donor bone marrow inoculum. A novel skin explant model, designed to take into consideration the immunobiological consequences of conditioning regimens on resident host cells, is proposed to advance our understanding of GVHD and serve as a potential prognostic tool when allogeneic recipient/donor combinations are being contemplated in the clinic. Within this review, specific emphasis is placed on the importance of defining the apoptotic machinery engaged in epidermal keratinocytes triggered by both conditioning regimens, and by host resident and recruited immunocytes and soluble mediators produced at sites of injury. The review is completed with a working model for cutaneous GVHD. Although the skin is highlighted because of its accessibility for clinical observations and serial sampling opportunities, lessons learned from studies of cutaneous GVHD are likely to provide valuable insights into GVHD occurring in the gastrointestinal tract, lung, and liver. With new insights designed to better predict and prevent GVHD and novel agents designed to treat GVHD, overcoming this current impediment to successful bone marrow transplantation should become increasingly feasible.

KEY WORDS

Apoptosis • Conditioning regimens • Cytokine storm • Dendritic (antigen presenting) cells • Skin explant assay

INTRODUCTION

Graft-versus-host disease (GVHD) is the principal toxicity of allogeneic bone marrow transplantation (BMT). Acute GVHD primarily affects 5 target organs: skin, gastrointestinal tract, liver, host lymphopoiesis, and lung. Clinically, acute GVHD of the skin results in an erythematous desquamating rash that characteristically involves the palms, soles, and trunk. Histopathology of involved skin may reveal focal or diffuse vacuolization of the basal cell layer (grade I); spongiosis and dyskeratotic keratinocytes (KCs) in close proximity to epidermal lymphocytes (aka satellite cell necrosis; grade II); necrotic KCs with pyknotic nuclei and eosinophilic cytoplasm, often with subepidermal cleft formation (grade III); and, finally, a complete loss of the epidermis (grade IV) [1]. Despite enormous strides in basic immunologic sciences, the prevention, diagnosis, and treatment of acute GVHD has remained unchanged in the past 2 decades.

The pathophysiology of acute GVHD in the skin can be considered as a 3-phase process, shown schematically in Figure 1. In phase 1, the conditioning regimen (irradiation, chemotherapy, or both) is postulated to initiate the damage of host tissues through-

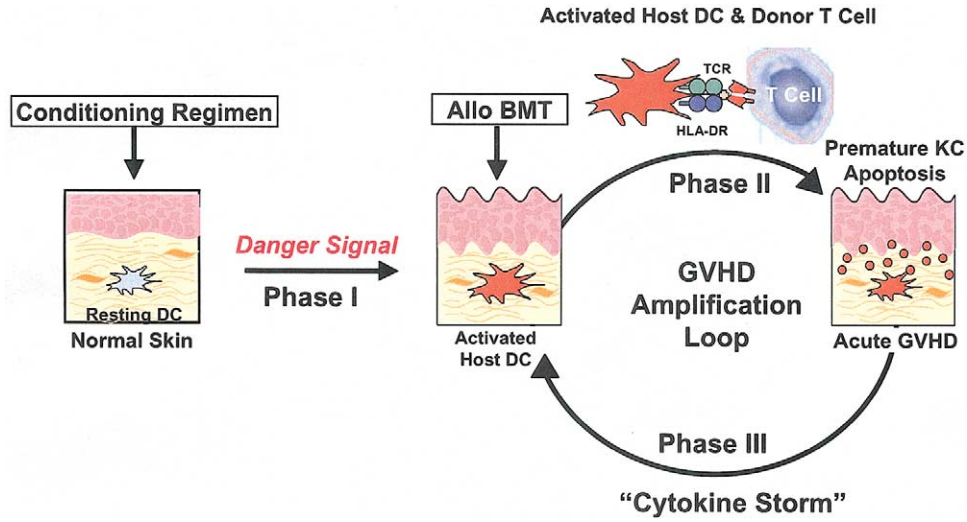

Figure 1. A 3-phase model is proposed for the pathogenesis of cutaneous GVHD. In phase 1, the conditioning regimen is postulated to create danger signals in skin by triggering either KC death or the release of cytokines and other soluble mediators that convert resting DCs to activated DCs. Expression of co-stimulatory molecules is accompanied by enhanced expression of major histocompatibility class I and II surface molecules. In phase 2, after BMT, donor T cells recognize alloantigens displayed on activated host DCs in skin, triggering the release of cytokines and other factors and culminating in premature apoptosis of KCs located predominantly in the rete ridges of the epidermis. We postulate that keratin-15^{+} KCs with stem cell–like properties are particularly vulnerable to the premature apoptotic reaction. In phase 3, a vicious cycle is established wherein initial KC cytotoxicity is accompanied by amplified cytokine secretion to produce "cytokine storm" that further enhances host DC activation and donor T-cell cytokine release, thus exacerbating KC cell death that ultimately spreads upward from the basal layer to include suprabasal layer KCs.

out the body and the production of danger signals, including secretion of the inflammatory cytokines tumor necrosis factor (TNF)–α and interleukin-1 [2,3], as well as heat shock proteins [4]. These danger signals activate resting dendritic cells (DCs) and enhance the expression of HLA-DR and various adhesion and co-stimulatory molecules [5,6]. Inflammatory cytokines may also stimulate the release of chemokines that can expedite the recruitment of donor T-cell effectors into the skin. The infusion of an allogeneic bone marrow inoculum initiates phase 2, wherein activated host DCs present alloantigens to donor T cells. In Figure 1, HLA-DR is both a marker of DC activation and the alloantigen recognized by the T-cell receptor on the donor T cells. Activated donor T cells further stimulate DCs through the engagement of co-stimulatory pathways such as CD40:CD40L, which in turn enhance T-cell stimulation and clonal expansion. In phase 3, the histopathologic changes of acute GVHD in the skin become apparent when KCs undergo premature apoptosis. Inflammatory cytokines such as TNF-α and interleukin-1 are major effectors of this process. These cytokines not only damage KC targets, but also perpetuate the activation of host DCs in a GVHD amplification loop between phase 2 and phase 3 ("cytokine storm"). This framework provides a basis for investigation of the precise molecular and cellular interactions that mediate acute GVHD and may help to develop new strategies to prevent, diagnose, and treat this intractable and toxic complication of allogeneic BMT.

PHASE 1: EFFECT OF CONDITIONING REGIMENS ON SUBSEQUENT DEVELOPMENT OF GVHD

The intensity of the conditioning regimen is a risk factor for acute GVHD. In patients with severe aplastic anemia, those who received irradiation had a higher risk of acute GVHD than those who received chemotherapy alone as induction therapy [7]. In patients with hematologic malignancies, higher doses of total body irradiation correlate with an increased risk of GVHD [8,9]. The role of ionizing radiation in the development of experimental GVHD was explored in one study that identified a synergistic effect in which preradiation exacerbated subsequent GVHD in murine skin [10]. Although several reports have documented the ability of chemotherapeutic drugs to affect the epidermis, the primary focus of these investigations has been to distinguish the cytotoxic effects of the drugs from GVHD [1,11-14]. Somewhat surprisingly, these studies did not move beyond this morphologic and diagnostic conundrum to probe the relationship between conditioning and the development of GVHD. As detailed in the following sections, we speculate that the effect of radiation is important not only for the damage caused to epidermal KCs, but also

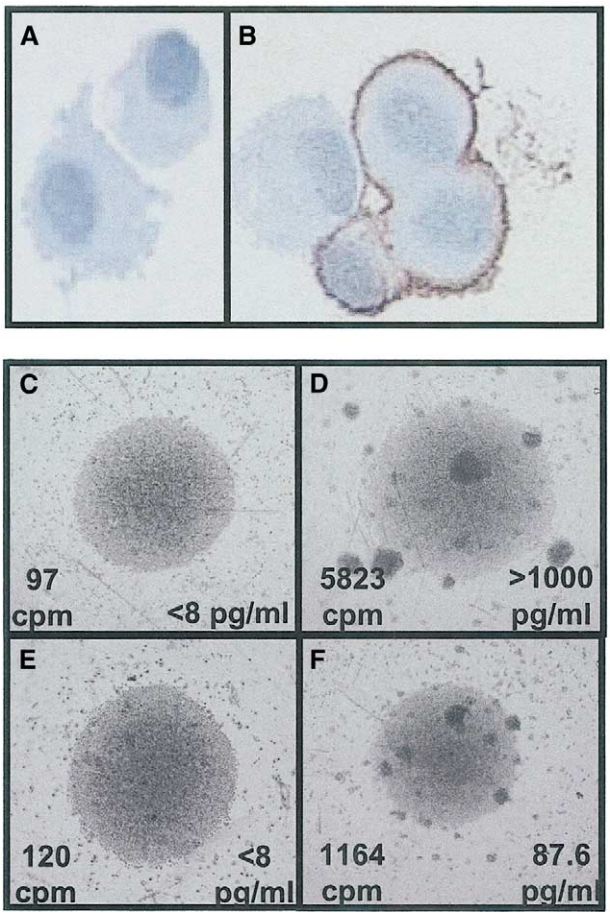

Figure 2. Novel skin explant model system designed to detect the immunobiological consequences of conditioning regimens on resident host cells. In this ex vivo model system, 6-mm punch biopsy samples of healthy human skin are placed in phosphate-buffered saline and exposed to 12 Gy of gamma irradiation as a single dose, followed by suspension in X-VIVO 15 medium for 18 hours at 37°C in a humidified incubator. Next, the tissue is minced and digested with a cocktail of enzymes including collagenase, hyaluronidase, and deoxyribonuclease for an additional overnight incubation at 37°C. After filtering through a mesh screen, total cells are counted and either used for cytospin preparations to detect HLA-DR on DCs (A and B) or added in triplicate wells to round-bottom 96-well plates to serve as stimulator cells (5×10^3 cells per well) and combined with allogeneic Ficoll-Hypaque–derived peripheral blood mononuclear cells (PBMCs; 1×10^5 cells per well). After 3 days of co-incubation, 50 μL of the 200-μL total reaction volume is removed for enzyme-linked immunosorbent assay to detect IFN-γ levels, and 2 days later, 1 μCi of ^{3}H-thymidine is added. The cells are harvested the following day to assess T-cell proliferation (C-F). A, Microscopic appearance of DCs produced from cytospin preparations of unirradiated skin that do not express HLA-DR. B, Immunohistochemical staining of cytospin preparations from irradiated skin in which DCs strongly and diffusely express HLA-DR. C, Negative control in which the phase microscopic appearance reveals a uniform cluster of round cells in the bottom of the well, with minimal ^{3}H-thymidine incorporation (97 cpm) and no significant IFN-γ production (28 pg/mL). D, Positive control in which 2 different PBMC donors were combined to generate a 2-way mixed lymphocyte reaction characterized by blastogenesis, as seen by colonies of activated appearing immunocytes by phase contrast microscopy. T-cell proliferation was confirmed by enhanced ^{3}H-thymidine incorporation (5823 cpm), and IFN-γ release was also high (>1000 pg/mL). E, Combination of unirradiated skin cells with allogeneic PBMC showing no significant T-cell proliferation (lack of colonizing formation and also 120 cpm of ^{3}H-thymidine incorporation) and no IFN-γ release (<8 pg/mL). F, Combination of epidermal and dermal cells derived from irradiated skin combined with allogeneic PBMC, in which phase contrast microscopy reveals blastogenesis confirmed by enhanced ^{3}H-thymidine incorporation (1164 cpm). This was accompanied by IFN-γ production (87.6 pg/mL).

for the activation of normal resting DCs in the dermis that provides the critical context for the alloreaction initiated by infusion of donor T cells in phase 2.

PHASE 2: HIGH-DOSE RADIATION ACTIVATES SKIN DCs AND CAUSES T-CELL STIMULATION IN MIXED LYMPHOCYTE REACTIONS

To explore the effect of conditioning regimens on antigen-presenting cells in human skin, we devised a skin explant model system in which punch biopsy samples of normal human skin were exposed to 12 Gy of irradiation (to simulate a BMT conditioning regimen) and then maintained in culture for 24 hours. After culture, cytospin preparations were stained for the activation marker HLA-DR as shown in Figure 2. In the absence of irradiation (panel A), DCs did not express HLA-DR. By contrast, after irradiation (panel B), there were numerous HLA-DR$^+$ cells, many of which had DC morphology, as assessed by light microscopy after immunohistochemical staining of cytospin preparations.

After the 24-hour incubation, biopsy specimens were minced, made into a single-cell suspension, and transferred into 96 round-bottom wells. Each well contained 5000 epidermal-derived cells (EDCs) that served as stimulators and 1×10^5 allogeneic peripheral blood mononuclear cells (PBMCs) that served as responders. As shown in Figure 2C-F, the ability of irradiated EDCs to activate allogeneic donor PBMCs was assessed by visual inspection for blastogenesis (day 3), by enzyme-linked immunosorbent assay measurement to detect interferon (IFN)–γ in the supernatant (day 3), and by thymidine incorporation to measure T-cell proliferation (day 7). Neither proliferation (97 counts per minute [cpm]) nor IFN-γ production (<8 pg/mL) was observed in the negative control wells (donor PBMCs alone), whereas the positive control (a bidirectional mixed lymphocyte reaction) fostered significant allogeneic T-cell responses (5923 cpm; IFN-γ >1000 pg/mL). EDCs from unirradiated skin were not capable of stimulating allogeneic T cells in these cultures (Figure 2E), whereas skin irradiated with 12 Gy (Figure 2F) caused significant T-cell proliferation and IFN-γ production (1164 cpm; IFN-γ 87.6 pg/

mL). Thus, high-dose radiation resulted in the activation of skin DCs and the subsequent stimulation of allogeneic T-cell responses. Whereas our studies used human skin and immunocytes, another group has observed that gamma irradiation can inhibit IFN-γ. Thus, additional studies are indicated to further evaluate the immunomodulatory effects of gamma irradiation on cell-mediated immune reactions in human skin.

Prior studies have suggested that factor XIIIa–positive dermal dendrocytes may be involved in the pathogenesis of disorders involving antigen presentation to T cells and dermal fibrosis [16]. Proof by association of increased activity of dermal dendrocytes has been provided by Yoo et al. [17] in an experimental murine GVHD model. Because some cases of acute cutaneous GVHD occur with only rare T cells present in lesional skin, it is possible that direct contact between cytotoxic T cells and KCs may not entirely explain the epidermal damage in GVHD. Furthermore, the distance between effector cells in the dermis, such as activated T cells and DCs, and epidermal targets, such as KCs, implies that soluble mediators released by immunocytes may mediate KC apoptosis. It is also possible that death ligands expressed by activated DCs, such as TNF-related apoptosis-inducing ligand, may contribute to KC apoptosis [18,19]. Of course, in animal models, investigators have also established that both $CD4^+$ and $CD8^+$ T-cell subsets possess cytotoxicity against epidermal cells in experimental GVHD [20-22].

PHASE 3: PREMATURE BASAL CELL KC APOPTOSIS IN GVHD

The process of apoptosis or programmed cell death is a natural event critical to homeostasis of all organ systems, including skin. To maintain a constant and functional epidermal thickness, the rate of cell proliferation must be matched to the rate of cell death and desquamation. The barrier function of the epidermis mandates that the timing of cell death be precisely coordinated in a 3-dimensional spatial context. The basal layer contains a subpopulation of cells similar to stem cells. They have a high proliferative potential, as indicated by retention of tritiated thymidine [23-25], are located in a protected bulge area of the hair follicle or interfollicular basal layer [26-28], and produce daughter cells that undergo a finite number of cell divisions before they differentiate [29]. Thus, KCs usually cannot prematurely undergo apoptosis in the basal layer (stratum spinosum) because this would perturb both epidermopoiesis and corneogenesis [30,31]. Many studies document the presence of various components of the apoptotic machinery in healthy human skin: Bcl-x [32], Fas [33,34], TNF-α [35], TNF receptor-1 [36], and activated caspase-3 [37]. Caspase-14 has also been documented in healthy murine skin [38].

The damage to epidermal KCs in GVHD of the skin occurs in a nonrandom fashion. Sale et al. [39] first observed in 1985 that KCs at the edge of the epidermis in the tips of rete ridges are the preferred target population in cutaneous GVHD. They suggested that these damaged KCs may represent stem cells. In a similar vein, Kim et al. [22] have recently documented vascular cell adhesion molecule-1 (CD106) expression on a distinctive subpopulation of KCs at the tips of retelike prominences in dorsal lingual epithelium that were highly susceptible to apoptosis in a murine model of acute GVHD. Basal cells in the rete ridges of normal human skin are positive for keratin-15 [40], a marker that has recently been linked to stem cells in skin by Lyle et al. [41]. In further studies, Whitaker-Menezes et al. [40] demonstrated selective apoptosis of keratin-15^+ KCs located at the tips of retelike prominences in a murine model of GVHD. These retelike prominences in the lingual epithelium of the mouse represent a microdomain very similar to rete ridges of human skin. The authors emphasized that the mode of KC cell death in GVHD was due to the induction of apoptosis, as opposed to necrosis.

These data suggest that GVHD causes premature activation of apoptotic machinery in basal KCs at the edge of the epidermis. In cells undergoing programmed cell death, the activation of caspase-3 occurs as a downstream event that links both extrinsic (death receptor–mediated) and intrinsic (mitochondrial- or DNA damage–mediated) apoptotic pathways. We reasoned that the presence of activated caspase-3 in a cell would not only identify it as apoptotic, but also indicate the activation of the apoptotic machinery involved in the premature demise of the targeted cell. We therefore stained GVHD skin with a commercially available antibody that recognizes only the activated, or cleaved, form of caspase-3 [42]. Apoptotic KCs identified by the uptake of the activated caspase-3 antibody, as well as by the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling assay in GVHD lesional skin, were preferentially located at the base of the rete ridges; this confirmed apoptosis as the mode of basal KC death in GVHD (B. Nickoloff, unpublished data). These experimental results support the notion that the conditioning regimen activates the apoptotic machinery in the basal KC layer, thereby making these cells more vulnerable to apoptosis when GVHD develops after BMT.

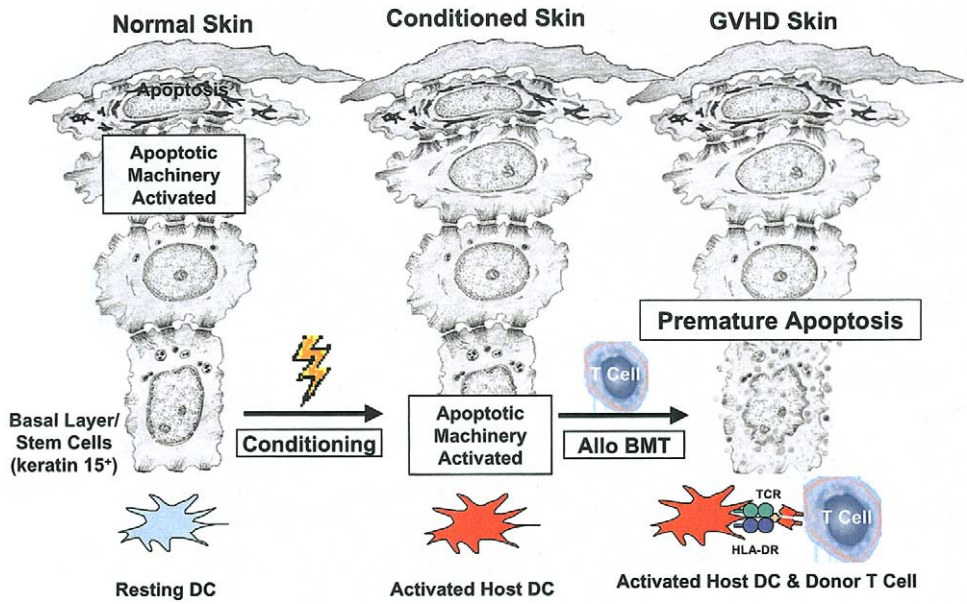

Figure 3. Working 3-phase model of acute cutaneous GVHD. In healthy human skin, the physiological apoptotic machinery is activated in the upper epidermal layers to produce the stratum corneum, and dermal DCs are in a resting state (blue). We postulate that after the patient is exposed to conditioning regimens (conditioned skin), apoptotic machinery is activated in the basal layer of keratin-15^+ KCs. In addition, danger signals from damaged KCs trigger activation of host DCs (red). In GVHD skin, donor T cells respond to activated host DCs after allogeneic (allo) BMT to generate a cytokine cascade that culminates in widespread premature apoptosis of epidermal KCs.

AN INTEGRATED HYPOTHESIS OF GVHD PATHOPHYSIOLOGY IN THE SKIN

Taken together, these data suggest a refined and integrated hypothesis for the mechanism of target cell apoptosis during acute GVHD (Figure 3). In phase 1, the conditioning regimen produces 2 distinct but related effects in the skin. First, it activates DCs in the dermis, priming them for an alloreaction that may occur when donor T cells are infused at the time of transplantation. Second, the apoptotic machinery in basal KCs is activated. During autologous BMT, when no alloreaction occurs, DC activation is self-limited, apoptotic machinery disengages, the basal KCs recover, and normal epidermopoiesis is restored. During an allogeneic BMT, donor T cells respond to the activated host DCs in the dermis. Inflammatory mediators such as TNF-α are produced that maintain engagement of the activated apoptotic machinery in basal KCs and ultimately contribute to premature apoptosis in this cellular compartment. These target cells thus participate in their own destruction through the activation of internal death programs, thus helping to explain the frequent lack of effector cells immediately adjacent to them.

It should be noted that GVHD can occur without a conditioning regimen. If donor T cells are sufficiently stimulated by host DCs, the T cells will further activate the DCs and perpetuate the alloreaction. In this case, the apoptotic machinery in basal KCs would not be activated prematurely; thus, target KC destruction would take longer to occur, require additional effector mechanisms, or both. The kinetics of the histologic change of GVHD in the skin would be altered, and the pathology itself might have different characteristics. Indeed, anecdotal evidence suggests that the manifestations of acute GVHD are different after reduced-intensity allogeneic BMT.

We believe that several aspects of our hypothesis can now be tested both in animal models and in the clinic. The skin is the most frequently biopsied and best studied target organ of acute GVHD. It represents an organized epithelial structure that renews itself and differentiates. Elucidation of the molecular processes that contribute to the destruction of specific subpopulations of target cells may lead to important insights not only for skin disease, but also for the other visceral targets of GVHD. Thus, a better understanding of life and death on the epidermal edge may ultimately facilitate the development of new strategies to diagnose, prevent, and treat this difficult complication of allogeneic BMT.

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