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Alloreactive Effector T Cells Require the Local Formation of a Proinflammatory Environment to Allow Crosstalk and High Avidity Interaction with Nonhematopoietic Tissues to Induce GVHD Reactivity

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Based on clinical observations that donor T cells specific for minor histocompatibility antigens (MiHA) ubiquitously expressed on both hematopoietic and nonhematopoietic cells were detected in patients showing evident graft-versus-leukemia/lymphoma (GVL) reactivity with no or limited coinciding graft-versus-host disease (GVHD), we hypothesized that nonhematopoietic tissues may be relatively unsusceptible to the cytotoxic effect of MiHA-specific T cells under normal, noninflammatory conditions. To test this hypothesis, we investigated the reactivity of alloreactive T cells specific for ubiquitously expressed MiHA against skin-derived primary human fibroblasts. We demonstrated that this reactivity was not merely determined by their antigen-specificity, but was highly dependent on adhesion molecule expression. ICAM-1 expression on the fibroblasts upregulated under proinflammatory conditions and induced during cross-talk with the T cells was demonstrated to be a crucial factor facilitating formation of high avidity interactions with the T cells and subsequent efficient target cell destruction. Furthermore, we provide supporting evidence for the role of ICAM-1 in vivo by demonstrating that ICAM-1 expression on nonhematopoietic target cells was dependent on the presence of infiltrating activated T cells, as was illustrated by restricted ICAM-1 expression at the sites of T cell infiltration in skin biopsies of patients with acute GVHD (aGVHD), by the absence of ICAM-1 expression in the same biopsies in areas without T cell infiltration and by the absence of ICAM-1 expression in biopsies of patients without GVHD independent of the presence of infiltrating nonactivated T cells. In conclusion, under noninflammatory conditions, nonhematopoietic tissues are unsusceptible to the GVHD reactivity of alloreactive T cells due to their inability to establish high avidity interactions.

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INTRODUCTION

Allogeneic stem cell transplantation (alloSCT) followed by cellular immunotherapy with donor-derived T cells can be a curative therapy for patients with hematological cancers [1,2]. After HLA-matched allo SCT, donor T cells can recognize patient cells as foreign, due to the expression of specific minor histocompatibility antigens (MiHA) [3-5]. Donor T cells recognizing MiHA specifically expressed on recipient hematopoietic cells, including the malignant cells, can mediate a therapeutic graft-versus-leukemia/lymphoma (GVL) effect [2,3,6-9]. However, donor T cells recognizing polymorphic peptides presented on normal nonhematopoietic tissues from the recipient may also mediate graft-versus-host disease (GVHD), which is the main cause of morbidity and mortality after alloSCT [10-12].

Surprisingly, high frequencies of circulating T cells expressing specific T cell receptors with high affinity for MiHA with ubiquitous expression in both hematopoietic and nonhematopoietic tissues have been detected in patients showing a strong GVL reaction in the

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absence of GVHD or with only limited GVHD [13-15]. These clinical data suggest that nonhematopoietic tissues are not always targeted by high affinity cytotoxic MiHA-specific T cells despite expression of the antigen. In contrast, in other studies, a clear correlation between the presence of T cells directed against ubiquitously expressed MiHA and the occurrence of GVHD has been suggested [16-18]. Intriguingly, Warren et al. [19] demonstrated that adoptive transfer of MiHA-specific donor T cells, even after in vitro depletion for reactivity against patient-derived fibroblasts, resulted in significant GVHD induction in vivo. Apparently, other factors besides expression of the MiHA also determine the susceptibility to T cell attack of nonhematopoietic target cells.

Efficient T cell-mediated cytolysis of (nonhematopoietic) target cells in vitro requires the formation of high avidity interactions between the immune effector cells and the target cells [20,21]. This avidity is determined by multiple factors, including the affinity of the T cell receptor of the effector cell, the level of expression of relevant peptide/MHC complexes on the target cell surface, and the expression of adhesion molecules, such as ICAM-1 [22-29]. In vivo, the local cytokine milieu most likely dictates the expression of molecules involved in the formation of a high avidity interaction between immune effector cells and nonhematopoietic target cells [24,30]. Investigations in mouse models demonstrated that conditioning and underlying diseases may damage host tissues resulting in production of so-called danger signals, including proinflammatory cytokines, chemokines, and amplified expression of adhesion molecules, MHC antigens, and costimulatory molecules on host tissue, thereby rendering these tissues more sensitive to T cell attack [11,31,32].

We hypothesized that in vivo the formation of a local proinflammatory cytokine milieu by locally activated T cells and subsequent sensitization of the surrounding nonhematopoietic cells to T cell attack determines whether or not GVHD is induced by T cells directed against ubiquitously expressed MiHA. In the current study, we demonstrate that the reactivity against skin-derived primary human fibroblasts of these T cells under noninflammatory, steady state conditions is low due to their inability to form a high avidity interaction. However, under proinflammatory circumstances, high avidity interactions are formed resulting in efficient targeting of the fibroblasts by the MiHA-specific T cells. ICAM-1 is demonstrated to be a key molecule mediating the high avidity interaction between T cells and nonhematopoietic target cells necessary for execution of their effector function, which is supported by the demonstration of co-localization of massive T cell infiltrates and upregulated ICAM-1 expression on the epithelial cells of the basal layer in skin biopsies of patients with acute GVHD (aGVHD). This study illustrates that in vivo reactivity of MiHA-specific T cells leading to GVHD cannot solely be predicted based on their antigen specificity. Under circumstances in which the magnitude of the immune response does not cause a local proinflammatory environment in the GVHD target tissues, targeting of MiHA with broad expression profiles may result in specific GVL reactivity without GVHD. On the other hand, absence of in vitro reactivity against patient nonhematopoietic tissues, as measured under noninflammatory conditions, does not guarantee protection against GVHD induction under clinical circumstances resulting in a local proinflammatory environment, which increases the susceptibility of cells in GVHD target tissues.

MATERIALS AND METHODS

Target Cells

After informed consent, primary human fibroblasts were generated from skin biopsies from patients or healthy donors. The skin biopsies were washed with PBS, minced, and transferred to 6-well culture plates containing low-glucose Dulbecco's modified Eagle medium (Lonza, Verviers, Belgium) supplemented with 10% FBS (Invitrogen, Breda, The Netherlands). Fibroblasts were cultured up to 90% confluency and then harvested using trypsin (Lonza, Verviers, Belgium) for 7 minutes at 37°C, followed by 2 washing steps. Stock samples were cryopreserved in liquid nitrogen. After thawing, the samples were reseeded at a concentration of 5000 cells/cm^2 and cultured again to 90% confluency, harvested, and reseeded. Experiments were performed using fibroblasts cultured for 5 to 20 passages. In specific experiments, fibroblasts were pretreated for 2 days with IFNgamma (IFNg; 200 IU/mL; Boehringer Ingelheim, Alkmaar, The Netherlands). In specific experiments, transduced fibroblasts were used. These fibroblasts were retrovirally transduced with pLZRS-constructs encoding HLA-A*0201, ICAM-1 (kindly provided by Dr. E. Hooijberg, VUMC, Amsterdam), or empty vector (mock), linked to the truncated human nerve growth factor receptor (NGFR) selection marker gene via an internal ribosome entry site sequence [33-35]. The identity of all constructs was verified by sequencing. Retroviral supernatants were generated with phoenix packaging (Φ-NX-A) cells, as previously described, and used for transduction of fibroblasts using recombinant human fibronectin fragments CH-296 (Lonza, Verviers, Belgium) [33].

Mock, ICAM-1, and HLA-A*0201-transduced fibroblasts were stained with NGFR-PE and purified by magnetic bead separation using anti-PE beads according to manufacturer's instructions (Milteny Biotec, Bergish Gladbach, Germany). Stable