

## BIOLOGY

# In Situ Detection of HY-Specific T Cells in Acute Graft-versus-Host Disease–Affected Male Skin after Sex-Mismatched Stem Cell Transplantation

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HY-specific T cells are presumed to play a role in acute graft-versus-host disease (aGVHD) after female-to-male stem cell transplantation (SCT). However, infiltrates of these T cells in aGVHD-affected tissues have not yet been reported. We evaluated the application of HLA-A2/HY dextramers for the in situ detection of HY-specific T cells in cryopreserved skin biopsy specimens. We applied the HLA-A2/HY dextramers on cryopreserved skin biopsy specimens from seven male HLA-A2<sup>+</sup> pediatric patients who underwent stem cell transplantation with confirmed aGVHD involving the skin. The dextramers demonstrated the presence of HY-specific T cells. In skin biopsy specimens of three male recipients of female grafts, 68% to 78% of all skin-infiltrating CD8<sup>+</sup> T cells were HY-specific, whereas these cells were absent in biopsy specimens collected from sex-matched patient–donor pairs. Although this study involved a small and heterogeneous patient group, our results strongly support the hypothesis that HY-specific T cells are actively involved in the pathophysiology of aGVHD after sex-mismatched stem cell transplantation.

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**KEY WORDS:** Minor histocompatibility antigen, Human, Pediatric, Antigen/peptide/epitopes, Hematopoietic stem cell transplantation

## INTRODUCTION

Acute graft-versus-host disease (aGVHD) is a life-threatening complication of allogeneic stem cell transplantation (SCT) mainly affecting the stem cell recipient's skin, liver, and/or gastrointestinal tract [1]. Matching the stem cell donor and recipient for HLA significantly reduces the risk for aGVHD and chronic GVHD [2]. In the HLA-matched SCT setting, the

development of GVHD is caused by donor T cells specific for ubiquitously expressed minor histocompatibility antigens [3], such as the Y chromosome-encoded HY antigens. Clinical results indicate that male recipients of female stem cells are at the greatest risk for GVHD [4].

Previous reports on the presence of HY-specific CD8<sup>+</sup> T cells in peripheral blood samples of adult male patients who developed aGVHD after sex-mismatched SCT [5,6] suggest the involvement of these cells in the pathophysiology of GVHD. This assumption is supported by results from in vitro studies using an ex vivo in situ skin explant model in which HY-specific cytotoxic T lymphocytes (T<sub>CTL</sub>) cause GVHD-like tissue destruction when added to male skin tissues expressing the relevant HY-presenting HLA class I molecules [7]. However, whether the presence of circulating HY-specific CD8<sup>+</sup> T cells detected before and after the onset of aGVHD reflects an active contribution of these cells to tissue destruction has remained unclear. We applied a dextramer-based staining technique to retrospectively analyze the in situ presence of HLA-A2/HY-specific CD8<sup>+</sup> T cells in archived cryopreserved skin biopsy specimens from 7 HLA-A2<sup>+</sup> male pediatric patients who developed aGVHD of the skin after

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**Table 1. Patient Characteristics**

Patient ID	Age at SCT, Years	Diagnosis	Conditioning Regimen	Donor Sex	Transplantation Type <sup>a</sup>	Donor Chimerism, % <sup>b</sup>	Clinical Onset of Acute Skin GVHD <sup>c</sup>	Time of Skin Biopsy (Grade) <sup>c</sup>	Minor H Antigen Mismatch in Graft-versus-Host Direction
UPN 567	4	CML	TBI, Cy, ATG	F	PBSC	ND	12	16 (I)	A2/HY, B60/HY, HA-1, HA-8, ACC-2
UPN 473	7	SAA	TAI, Cy, ATG	F	BM	100%	22	24 (I-II)	A2/HY
UPN 463	6	X-ALD	Bu, Cy, ATG	F	BM	ND	13	14 (I-II)	A2/HY, B8/HY
UPN 574	4	SAA	Cy, Treo, ATG	M	BM	100%	14	16 (II)	-
UPN 577	7	MDS RAEBII	Bu, Cy, Mel, Cam1H	M	PBSC	ND	12	14 (II)	HLA- 2/LB-ADIR-1
UPN 501	7	X-LPD	Bu, Cy, ATG	M	BM	100%	14	18 (I)	HLA-A2/LB-ADIR-1
UPN 520	15	ALL	TBI, Cy, VPI6, ATG	M	BM	100%	35	39 (II)	HLA-A2/HA-8

CML indicates chronic myelogenous leukemia; SAA, severe aplastic anemia; X-ALD, X-linked adrenoleukodystrophy; MDS RAEBII, myelodysplastic syndrome (refractory anemia with excess number of blasts); X-LPD, X-linked lymphoproliferative disease; ALL, acute lymphoblastic leukemia; TBI, total body irradiation; TAI, thoracoabdominal irradiation; Cy, cyclophosphamide; ATG, anti thymocyte globulin; Bu, busulfex; Treo, treosulphan; Mel, melphalan; Cam1H, Campath (alemtuzumab); VPI6, etoposide; PBSC, G-CSF mobilized peripheral blood stem cells; BM, bone marrow; ND, not determined. <sup>a</sup>T cell depletion of the graft through antibody-induced T cell rosetting (UPN 463), purified CD34 with T cell add-back (UPN 567), or Campath "in the bag" (UPN 577).

<sup>b</sup>Percentage donor chimerism was determined by VNTR analysis (PowerPlex 16) on PBMCs collected between 21 and 28 days post-SCT.

<sup>c</sup>Days after hematopoietic stem cell graft infusion, followed by the Lerner GVHD grading [11] in parentheses.

undergoing allogeneic HLA-matched unrelated SCT with a male or a female donor.

## METHODS

### Study Participants

All skin biopsy samples analyzed in this study were derived from HLA-A2<sup>+</sup> male individuals and were collected after written informed consent was obtained in accordance with the Declaration of Helsinki. Skin samples were collected from five healthy adult volunteer donors and from seven pediatric patients who underwent allogeneic SCT for the treatment of various hematologic and nonhematologic disorders (Table 1). Patient selection was based on the expression of HLA-A2 by patient and donor, the development of histologically confirmed aGVHD [8] involving the skin and the availability of a cryopreserved skin biopsy specimen collected before the initiation of first-line treatment with corticosteroids. All but two patients (UPN 567 and UPN 577) received a 10/10-matched (HLA-A, -B, -C, -DQ, and -DR) stem cell graft from an unrelated donor. Three of the seven male patients (UPN 567, UPN 473, and UPN 463) received a stem cell graft from a female donor. The use of peripheral blood mononuclear cells (PBMCs) and skin samples for the underlying study was approved by Leiden University Medical Center's Medical Ethics Committee.

### Multimeric HLA Class I Peptide Complexes

Conventional allophycocyanin (APC)-conjugated HLA-A2 tetramers and phycoerythrin (PE) conjugated HLA-A2 dextramers (Immudex, Copenhagen, Denmark), both containing the HLA-A2-restricted HY peptide FIDSYICQV (designated as HLA-A2/HY tetramers or HLA-A2/HY dextramers), as well as control PE-conjugated HLA-A2 dextramers containing the influenza peptide GILGFVFTL (HLA-A2/flu

dextramers) were prepared as described previously [6,9,10]. The specificity and sensitivity of these HLA-A2/peptide multimers were routinely determined by fluorescent activation cell sorting analysis (Supplementary Figure S1), as described previously [6,9].

### Immunofluorescent Staining and Confocal Laser Scanning Microscopy Analysis of Cryopreserved Skin Explant Tissue and aGVHD-Affected Skin Tissue

Before cryopreservation, skin explant assays were performed with freshly obtained healthy male skin biopsy specimens that were coincubated in vitro for 72 hours at 37°C and 5% CO<sub>2</sub> with 1 × 10<sup>6</sup> HY-specific T<sub>CTL</sub>, as described in detail elsewhere [7,11]. Control skin biopsy specimens, prepared from the same healthy donors, were incubated with HLA-A2/HA-1-specific T<sub>CTL</sub>. Of note, the latter T cells infiltrate human skin in the presence of HA-1<sup>+</sup> dendritic cells [12], but unlike the HY-specific T<sub>CTL</sub>, HA-1-specific T<sub>CTL</sub> do not cause GVHD-like tissue destruction [7,12]. Cryosections (6 μm) were prepared from both snap-frozen skin explant tissue and cryopreserved aGVHD-affected skin biopsy specimens. After acetone fixation, the sections were stained at 4°C with PE-conjugated HLA-A2/HY dextramers or control PE-conjugated HLA-A2/Flu dextramers, followed by appropriately diluted rabbit anti-PE antibody (Biogenesis, Poole, United Kingdom) and cyanin 3-labeled goat anti-rabbit F(ab)<sub>2</sub> antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). All cryosections analyzed in this study (from volunteer donors and from SCT patients) were simultaneously stained with FITC-conjugated CD8 antibody (Beckman Coulter, Woerden, The Netherlands) [11]. Combined immunofluorescent staining using antibodies specific for CD3 (Dako, Glostrup, Denmark) CD4 (Neomarkers via Immunologic, Duiven, The Netherlands), CD8 (Novocastra

via Leica Microsystems B.V., Rijswijk, The Netherlands) or CD8 (Beckman Coulter)/CD45RO (Dako)/granzyme B (Sanquin, Amsterdam, The Netherlands) was performed as described previously [11,13]. All sections were mounted with vectashield (Vector Laboratories, via Reactolab SA, Servion, Switzerland) and analyzed on a Leica TCS SP confocal laser scanning system (Leica Microsystems B.V.).

Images were collected sequentially using a 40 × numerical aperture 1.4 objective. Color photographs were generated as electronic overlays. For each patient except UPN 520, between four and 18 consecutive cryosections were scored for the presence of CD8<sup>+</sup> HLA-A2/HY dextramer-negative and CD8<sup>+</sup> HLA-A2/HY dextramer-positive T cells. A single section prepared from a biopsy specimen from UPN 520 was scored in a similar way. Each section consisted of five to seven confocal images of 512 μm × 512 μm × 6 μm (W × L × H) each, covering the complete section. All positively staining cells were individually analyzed while scanning in the z-axis direction to obtain a three-dimensional confocal image window, to exclude inclusion of noncellular structures in the total number of positive cells counted in nearly the complete biopsy.

### Statistical Analysis

In each patient, the mean number of HLA-A2/HY dextramer-positive CD8<sup>+</sup> T cells per cryosection was calculated. Differences between the mean values observed in the biopsy specimens of sex-matched and sex-mismatched recipients were analyzed by a two-tailed unpaired *t*-test using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, CA).

## RESULTS

### Onset of aGVHD Coincides with an Increase in Peripheral Blood Lymphocytes

All patients exhibited the first clinical symptoms of aGVHD in the skin between 12 and 35 days after SCT (Table 1). Results on post-SCT peripheral blood lymphocyte recovery were available in six of the seven patients (Supplementary Figure S2). The missing data for UPN 577 were due to early death, at day +15 after SCT. Although total peripheral blood lymphocyte counts were not in the range of those obtained before SCT (2745 ± 809/μL), all six patients displayed an increase in absolute numbers of lymphocytes at or shortly after the day of skin biopsy collection (range, 80-1073/μL). CD4<sup>+</sup> and CD8<sup>+</sup> T cell subset analysis was performed on blood samples collected shortly before and after onset of aGVHD from UPN 567, UPN 574, UPN 501, and UPN 520. In line with data on total lymphocyte counts measured in parallel, both CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts were increased after the onset of aGVHD. Sufficient numbers of PBMCs

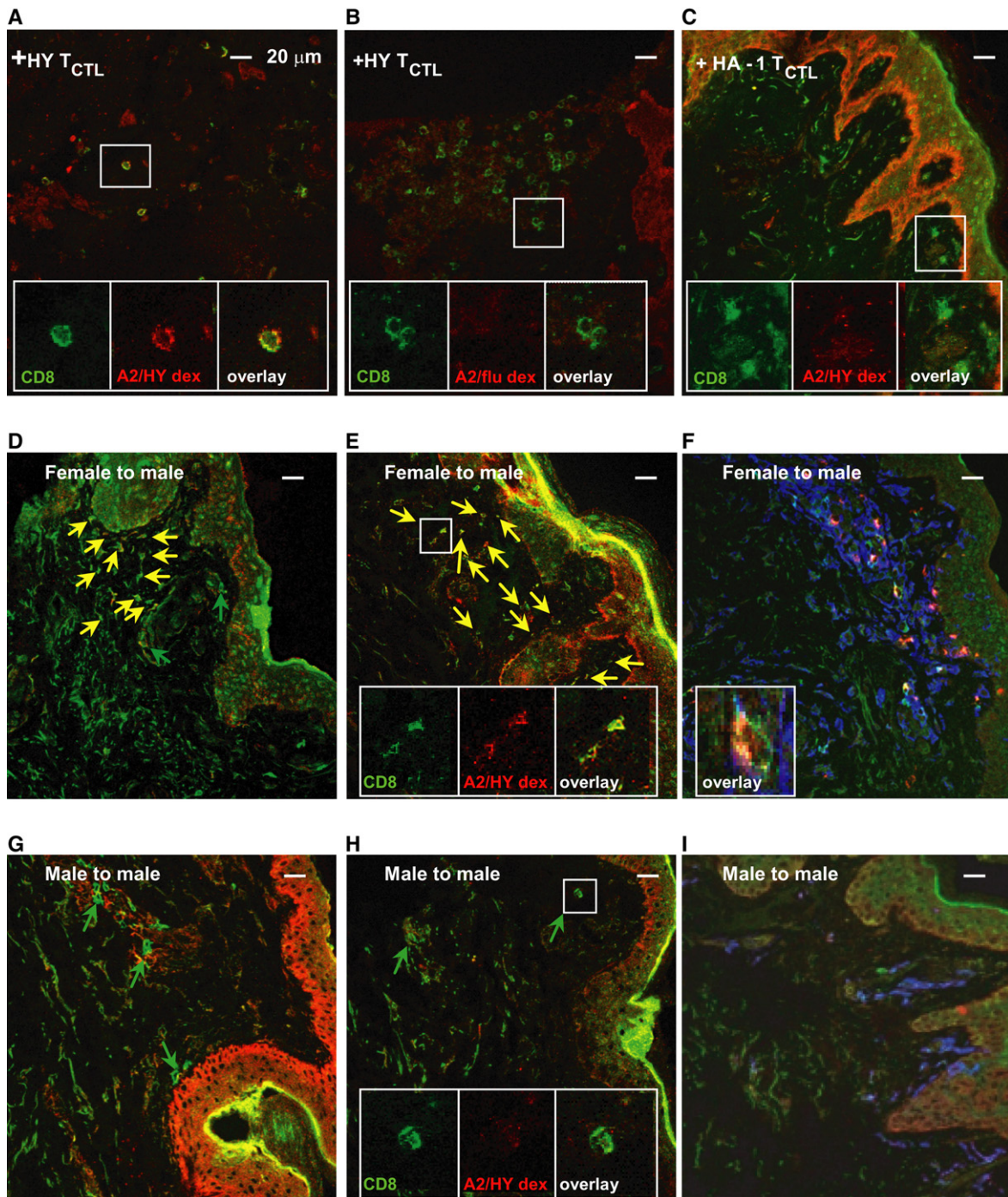
from UPN 567 and UPN 473 (both male recipients of a female stem cell graft) were available for additional HLA-A2/HY tetramer analysis performed at 2 and 4 weeks (UPN 567) or 4 and 5 weeks (UPN 473) after SCT. The numbers of HY-specific T cells in CD8<sup>+</sup> cell fractions were below the detection limit in all four PBMC samples analyzed (data not shown). Chimerism analyses performed on PBMC samples collected 21 to 28 days after SCT revealed complete donor origin in four (UPN 473, UPN 574, UPN 501, and UPN 520) of six patients (Table 1).

### Validation of HLA-A2/HY Dextramers for in Situ Detection of HY-Specific T Cells in Cryopreserved Skin Explant Tissue

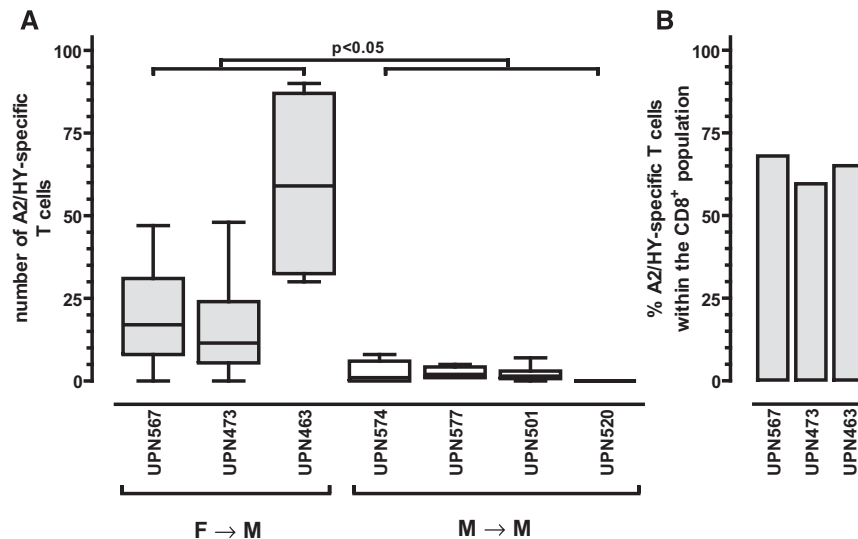
Failure to detect circulating HY-specific CD8<sup>+</sup> T cells after the onset of aGVHD in lymphopenic sex-mismatched recipients does not exclude their possible presence in aGVHD-affected tissues. Thus, we analyzed archived frozen skin biopsy specimens from our patient cohort. Because the conventional HY tetramers do not stain frozen biopsy specimens [11], we first validated HLA-A2/HY dextramers on frozen sections prepared from ex vivo in situ skin explant tissue for their selective staining capacities [7,11]. To this end, HLA-A2/HY-specific T<sub>CTL</sub> or control HLA-A2/HA-1-specific T<sub>CTL</sub> were added exogenously to fresh skin tissue derived from HLA-A2<sup>+</sup> healthy male volunteer donors. Similar to conventional HLA-A2/HY tetramers [11], HLA-A2/HY dextramers stained skin-infiltrating HY-specific T<sub>CTL</sub> when applied to viable skin explant tissue that was snap frozen after the addition of either staining reagent (data not shown). However, in the clinical setting of pediatric SCT, obtaining fresh tissue biopsy specimens for such experiments is difficult. Therefore, we analyzed the capacity of HLA-A2/HY dextramers to stain HY-specific T<sub>CTL</sub> when applied to already cryopreserved tissue. HLA-A2/HY dextramers, in contrast to HLA-A2/HY tetramers, were able to stain HY-specific T<sub>CTL</sub> when applied to cryosections prepared from snap-frozen skin explant tissue (Figure 1A). Control HLA-A2/Flu dextramers did not label skin-infiltrating HY-specific T<sub>CTL</sub> (Figure 1B). Furthermore, HLA-A2/HY dextramers did not stain cryosections preincubated with HA-1-specific T<sub>CTL</sub> (Figure 1C). Collectively, these results show the specificity and applicability of HLA-A2/HY dextramers to visualize HY-specific CD8<sup>+</sup> T cells in cryopreserved skin tissue collected for clinical evaluation.

### Presence of HY-Specific CD8<sup>+</sup> T Cells in aGVHD-Affected Skin collected from Male Recipients of Female Stem Cell Grafts

Historically collected cryopreserved skin biopsy specimens from seven pediatric patients with



**Figure 1.** In situ staining of skin-infiltrating  $CD8^+$  T cells reveals the presence of both granzyme B<sup>+</sup> and HLA-A2/HY dextramer-positive cells in aGVHD-affected skin tissue obtained after female-to-male SCT. Skin explant tissue derived from healthy male HLA-A2<sup>+</sup> volunteer donors was used to validate the sensitivity and specificity of HLA-A2/HY dextramers (red) for combined in situ labeling of skin-infiltrating  $CD8^+$  T cells (green). These skin segments were incubated with either HY-specific  $T_{CTL}$  (A and B) or control HA-I-specific  $T_{CTL}$  (C) before cryopreservation and immunofluorescent labeling. Double-labeled cells, which are HY-specific  $CD8^+$  T cells, become yellow in the overlay illustrations, as depicted by the representative cell in the lower right overlay insert of (A). Single-labeled cells (ie,  $CD8^+$  cells) remain green in the overlay pictures, as depicted by the representative cells in the lower right insert of (B) and (C). (D and E) Combined immunofluorescent staining of cryosections prepared from aGVHD-affected skin biopsy specimens obtained from two male recipients of a female graft (D, UPN 473; E, UPN 567). The cryosections were incubated with HLA-A2/HY dextramers (red) and CD8 antibodies (green). Yellow arrows indicate colocalization of HLA-A2/HY dextramers and CD8 antibodies on the same cell. (F) Combined CD8 (green), CD45RO (dark blue), and granzyme B (red) staining in a cryosection prepared from the same individual as shown in (E). (G and H) The identical staining procedure as described in (D) and (E) but applied to cryosections obtained from two male recipients of a male graft (G, UPN 501; H, UPN 574). Green arrows indicate  $CD8^+$  cells not expressing the T cell receptor that specifically binds HLA-A2/HY dextramers. The higher magnification of a representative single-labeled cell in (E) depicts a  $CD8^+$   $T_{CTL}$  that is not HY-specific. Note that keratinocytes located at the dermal-epidermal junction may display some nonspecific red staining when incubated with dextramer preparations. (I) Combined CD8 (green), CD45RO (dark blue), and granzyme B (red) staining in a cryosection prepared from the same individual shown in (E).



**Figure 2.** Acute GVHD after female-to-male SCT is associated with significantly higher numbers of skin-infiltrating CD8<sup>+</sup> T cells containing a high percentage of cells specific for HY. (A) Total numbers of CD8<sup>+</sup> cells (left) and HLA-A2/HY dextramer-positive CD8<sup>+</sup> cells (right) were counted in 1-18 serial cryosections prepared from the same cryopreserved skin biopsy specimen collected shortly after clinical onset of aGVHD and before the start of first-line treatment. All positively staining cells were individually analyzed while scanning in the z-axis direction to obtain a three-dimensional confocal image window, to avoid inclusion of noncellular structures in the total number of positive cells counted in nearly the complete biopsy specimen. For each individual patient, the box-and-whisker plot shows the mean number  $\pm$  SD of cells counted per section as indicated on the y-axis. The upper and lower ends of the boxes represent the upper and lower quartiles, respectively. Whiskers represent the lowest and highest observations. The solid line represents the median. \* $P < .05$ . (B) Percentage of HLA-A2/HY-specific T cells within the CD8<sup>+</sup> population in the three female-to-male SCT recipients.

confirmed aGVHD of the skin were available for this analysis. The validated HLA-A2/HY dextramers were used to analyze these cryosections. The presence of HY-specific T<sub>CTL</sub>, visualized by HLA-A2/HY dextramer and CD8 costaining cells, was found in two of the three male recipients of a female stem cell graft (Figure 1D and E). Higher-magnification images of two double-positive cells are shown in the insert at the bottom of Figure 1E. Of note, skin-infiltrating HY-specific T cells were observed in biopsy specimens collected as early as 12 to 13 days after sex-mismatched SCT (UPN 463 and UPN 567). These T cells accumulated at the same location as HY-specific CTL clones applied in the skin explant assay [7], that is in the dermis as well as just below the dermal-epidermal junction (Figure 1D and E). At the same location, granzyme B-coexpressing CD8<sup>+</sup> cells were visualized (Figure 1F), illustrating that aGVHD skin-infiltrating CD8<sup>+</sup> T cells are “licensed to kill” and are not innocent bystanders. Unfortunately, the only available HLA-A2/HY dextramers and the granzyme B-specific antibody were both conjugated with PE, hindering the analysis of coexpression of these two markers by the same CD8<sup>+</sup> T cell.

CD8<sup>+</sup> HLA-A2/HY dextramer-positive T cells were not detected in any of the biopsy specimens obtained from male recipients of a male stem cell graft (Figure 1G and H). These cryosections contained only a few CD8<sup>+</sup> T cells, as exemplified by the enlarged single positive green cell shown at the bottom of Figure 1H. But these CD8<sup>+</sup> cells were not

HY-specific and did not express granzyme B, in sharp contrast to the skin-infiltrating CD8<sup>+</sup> cells shown in Figure 1F. Control staining of serial sections with HLA-A2/Flu dextramers revealed no positive signal in any of the biopsy tissues tested (data not shown).

Quantification of the CD8<sup>+</sup> T cell infiltrates was performed on skin biopsy specimens from all seven patients (Figure 2). Significantly higher numbers of CD8<sup>+</sup> HY-specific T cells infiltrated the skin after sex-mismatched SCT than after sex-matched SCT ( $P < .05$ ) (Figure 2A). In contrast to the biopsy specimens obtained after sex-matched SCT, high proportions (68%-78%) of aGVHD skin-infiltrating CD8<sup>+</sup> cells in the male recipients of a female graft were HY-specific (Figure 2B).

## DISCUSSION

HY-specific T cells may contribute to the development of GVHD in male recipients of female hematopoietic stem cell grafts [14]. Although numerous clinical studies have shown the influence of sex mismatching on SCT outcome, the in situ presence of HY-specific T cells in GVHD-affected tissues has not been shown until now. The available tetrameric HLA HY peptide complexes do not stain infiltrated T cells in cryopreserved tissues.

In this study, we first validated the use of dextrameric HLA-A2/HY peptide complexes using an ex vivo skin explant model [7] with cryopreserved

skin tissues of healthy male individuals. The newly developed dextrameric HLA-A2/HY peptide complexes allowed staining and analysis of infiltrating T cells in cryopreserved tissues. These results thus allowed us to perform a retrospective analysis of earlier stored clinical GVHD samples.

For the first time, using dextrameric HLA-A2/HY peptide complexes, we visualized HY-specific CD8<sup>+</sup> T cells in aGVHD-affected tissue obtained from male recipients of female stem cell grafts. Application of the PE-labeled dextrameric HLA-A2/HY complexes combined with CD8-specific antibodies showed skin-infiltrating HY-specific T cells in cryopreserved skin biopsy specimens obtained shortly after clinical manifestation of aGVHD in three male pediatric recipients of a female stem cell graft. Despite the first signs of lymphocyte recovery in the circulation, HY-specific T cells were not detected in peripheral blood samples collected from two of these three SCT recipients (UPN 567 and UPN 473) at 2 to 5 weeks after SCT. In previous work, we reported the presence of HY-specific T cells in the peripheral blood of adult male SCT recipients who developed aGVHD after receiving a non-T cell-depleted bone marrow graft from a female donor [6]. These seemingly contradictory results might be related to the significant differences in applied SCT protocols between pediatric and adult patients, such as composition of the applied stem cell grafts and the pre- and post-SCT applied immune suppression regimens. Of note, severe lymphopenia in the first few weeks after SCT, in combination with the limited volume of peripheral blood routinely collected from pediatric SCT patients, has hampered reliable HLA-A2/HY tetramer analyses in PBMCs. Thus, our analysis was limited, as two of the three pediatric male recipients of female grafts could be analyzed. Note, however, that we found a relatively high number of HY-specific T cells migrating to the skin during the early stage of aGVHD. Whether or not this clarifies the absence of minor HY-specific T cells in the peripheral blood is a subject for extensive future analysis.

Given that TCR stimulation-induced cell cycle progression is required for the induction of granzyme B-containing cytolytic granules in both naïve and antigen-experienced T<sub>CTL</sub> [15], the presence of highly granzyme B<sup>+</sup> CD8<sup>+</sup> in aGVHD-affected skin suggests that these presumably cytolytic T cells are activated. As shown in Figure 1F, activated T<sub>CTL</sub> ultimately release their cytolytic granules in the dermis or at the dermal-epidermal junction. At the same location, GVHD-like tissue destruction occurs in vitro when HY-specific T<sub>CTL</sub> clones are applied in the skin explant assay [7]. We previously demonstrated that HLA-A2/HY-specific T<sub>CTL</sub> clones lyse IFN- $\gamma$ -activated HLA-A2<sup>+</sup> epidermal keratinocytes in vitro [16]. In vivo, IFN- $\gamma$  may be derived from the activated T<sub>CTL</sub> themselves and/or from activated, that is,

CD45RO expressing CD4<sup>+</sup> T cells present in the same dermal infiltrates (Figure 1F).

The early appearance of HY-specific CD8<sup>+</sup> T cells in aGVHD-affected skin raises the question whether these T cells arise from naïve or memory T cells, both of which are likely cotransferred along with the female hematopoietic stem cell graft into the male patient. Although we were not able to analyze pre-SCT collected female donor PBMCs in the present study, it should be noted that pregnancy can induce the generation and long-term persistence of minor histocompatibility antigen-specific T<sub>CTL</sub>, including T cells specific for HY [17-19]. It is possible that such memory-type minor histocompatibility antigen-specific T cells are also present in hematopoietic stem cell products prepared from parous female donors. On reencountering the relevant minor histocompatibility antigen in the stem cell recipient, these T cells may contribute to the development of aGVHD. Prospective analyses of the presence of cytolytic type T cells specific for ubiquitously expressed minor histocompatibility antigens in female stem cell products may identify SCT patient-donor pairs at risk for aGVHD.

In conclusion, the present study provides the first indication that HY-specific T cells are actively involved in the development of aGVHD after sex-mismatched SCT. The size of our study population was small and the patient population relatively heterogeneous, however, and further studies on larger and more homogeneously treated patients are needed to evaluate the clinical significance of our observations.

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**Authorship Statement:** Eric Spierings, Florry A. Vyth-Dreese, Maarten J. D. van Tol, and Els Goulmy designed the research; Jørgen Schøller, Stan Pavel, and R. Maarten Egeler provided crucial reagents and tissue specimens; Yeung-Hyen Kim, Claudia M. J. M. Faaij, Ellen Schrama, Trees A. M. Dellelijn, and Astrid G. S. van Halteren performed experiments and collected data; Yeung-Hyen Kim, Eric Spierings, Claudia M. J. M. Faaij, Florry A. Vyth-Dreese, and Astrid G. S. van Halteren analyzed results and prepared figures; and Astrid G. S. van Halteren, Eric Spierings, Maarten J. D. van Tol, and Els Goulmy drafted the manuscript.

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## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2011.10.038.

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