Blockade of Granzyme B Remarkably Improves Mucocutaneous Diseases with Keratinocyte Death in Interface Dermatitis

TO THE EDITOR

Lichenoid tissue reaction/interface dermatitis (LTR/IFD) is a pattern of skin inflammation characterized pathologically by infiltration of the skin basement membrane by auto-aggressive T cells, causing keratinocyte death (Sontheimer and Gilliam, 1981). A wide spectrum of skin disorders exhibit this set of histological features and mucocutaneous lesions, including acute graft-versus-host disease (aGVHD). Previous clinical studies on disorders with LTR/IFD have described that CD8+ T cells mainly infiltrate the blistered epidermis and their number increases concomitantly with disease progression. The infiltrating CD8+ T cells were distributed around apoptotic keratinocytes and express PRF1 and granzyme (Gzm) B (Correia et al., 2001; Jungell et al., 1989; Paller et al., 1988; Takata et al., 1993). Serum levels of soluble FasL produced by CD8+ T cells were also reportedly increased in patients with aGVHD (Liem et al., 1998). Studies with allogeneic bone marrow transplant murine models also supported these clinical observations (Baker et al., 1996; Braun et al., 1996; Graubert et al., 1996). We particularly evaluated the impact of PRF1/Gzm and Fas/FasL pathways on LTR/IFD.

We used chicken ovalbumin (OVA) transgenic mice, in which keratinocytes express membrane-bound OVA under the control of a keratin 14 promoter (K14-mOVA mice). K14-mOVA mice develop aGVHD-like mucocutaneous disease (Supplementary Figure S1 online) and weight loss was observed after transfer of transgenic CD8+ T cells expressing OVA-specific T-cell receptor (from GFP transgenic OT-I mice) (Shibaki et al., 2004). Weight loss during late-phase reflects the severity of mucocutaneous lesions causing difficulty in food intake. Hematoxylin and eosin staining of ear specimens showed LTR/IFD (Supplementary Figure S2 online). Immunofluorescence staining with anti-GFP antibodies revealed infiltration of GFP+ OT-I cells in the epidermis and dermis, which resulted in a number of dead keratinocytes revealed by TUNEL assay (Supplementary Figure S2). The disease is mediated by CD8+ T cells (Supplementary Figure S3 online).

To investigate the roles of cytotoxic molecules on LTR/IFD development, we observed wild-type (WT), PRF1+/−, GzmA−/−, GzmB−/−, and Fasl−/−OT-I cell–transferred recipient K14-mOVA mice. PRF1+/−/OT-I cell– and GzmB−/−/OT-I cell recipients did not develop mucocutaneous lesions, whereas GzmA−/−OT-I cell recipients developed milder lesions than those in WT OT-I cell recipients at 14 days post-OT-I cell transfer (Figure 1a, 1b). PRF1+/−/OT-I cell recipients and GzmB−/−/OT-I cell recipients completely recovered their body weights, whereas GzmA−/−OT-I cell recipients presented a late-phase weight loss similar to WT (Figure 1c). Hematoxylin and eosin–stained ear specimens of PRF1+/−/OT-I and GzmB−/−/OT-I cell recipients did not show LTR/IFD without dead keratinocytes and mild infiltration

Abbreviations: aGVHD, acute graft-versus-host disease; Gzm, granzyme; LTR/IFD, lichenoid tissue reaction/interface dermatitis; OVA, ovalbumin; WT, wild-type

Accepted manuscript published online 22 March 2018; corrected proof published online 26 May 2018 © 2018 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.
Figure 1. PRF1 knockout (PRF1<sup>−/−</sup>) and granzyme B knockout (GzmB<sup>−/−</sup>) CD8 T cells that completely lost their cytotoxicity cannot induce acute graft-versus-host disease–like mucocutaneous lesions. (a, b) Clinical manifestations (a) and skin scores (b) of K14-mOVA mice at 14 days after transfer of wild-type (WT), PRF1<sup>−/−</sup>, GzmB<sup>−/−</sup>, GzmA<sup>−/−</sup>, and FasL<sup>−/−</sup> OT-I cells. (c) Courses of weight change. n = 10. (d) Hematoxylin and eosin (HE) staining (at 14 days post–OT-I cell transfer), immunofluorescence staining for GFP (9 days) and TUNEL assay (at 9 days) for ear skin samples. White and blue broken lines indicate epidermal borders. Scale bar = 50 μm. (e) Percentage of the length of lesions with LTR/IFD per whole-field area of HE-stained samples. (f) Percentages of TUNEL-positive cells in the epidermis counted in a field at ×200 magnification. (g, h) GFP<sup>+</sup> OT-I cells in the epidermis (g) and dermis (h).
Figure 2. Mucocutaneous disease with LTR/IFD in K14-mOVA mice was remarkably improved after serpina3n treatment. (a) Administration protocols. (b) Courses of weight change. n = 6. (c) Clinical manifestations at 5 and 14 days post-OT-I cell transfer. (d) Course of skin scores. n = 6. (e) HE staining (at 14 days post-OT-I cell transfer), immunofluorescence staining for GFP (at 9 days) and TUNEL assay (at 9 days) for ear skin samples. White and blue broken lines indicate epidermal borders. Scale bar = 50 μm. (f) Percentage of the length of lesions with LTR/IFD per whole-field area of HE-stained samples. n = 6. (g, h) GFP+OT-I cells in the epidermis (g) and dermis (h) counted in a field at ×200 magnification. n = 6. (i) Percentage of TUNEL-positive cells in the epidermis, counted in a field at ×200 magnification. n = 6. Each bar and curve represents the mean ± standard deviation. *P < 0.05, **P < 0.01. All data are representative of duplicate independent experiments. HE, hematoxylin and eosin; LTR/IFD, lichenoid tissue reaction/interface dermatitis; OVA, ovalbumin.
by GFP⁺ OT-I cells limited only to the dermis, whereas GzmA⁻/⁻OT-I cell recipients developed moderate mucocutaneous lesions with significantly decreased affected areas, numbers of infiltrating GFP⁺ OT-I cells and dead keratinocytes compared to those in WT OT-I cell recipients (Figure 1d–1h). In contrast, FasL⁻/⁻ OT-I cell recipients developed mucocutaneous lesions with histological changes and weight loss as severe as those observed in WT OT-I cell recipients (Figure 1a–1h).

To investigate the characteristics of PRF1⁻/⁻, GzmA⁻/⁻, and GzmB⁻/⁻ OT-I cells, we performed cytotoxic release assays revealing that PRF1⁻/⁻ OT-I cells and GzmB⁻/⁻ OT-I cells completely lost their cytotoxicity, whereas GzmA⁻/⁻ OT-I cells lost it partially (Figure 1i). In vivo proliferation assays showed that proliferation of the three types of OT-I cells was not impaired in K14-mOVA mice (Figure 1j). Moreover, the expression levels of cell surface activation markers and production of inflammatory cytokines (TNF-α and IFN-γ) in the 3 types of transferred OT-I cells were not different from those in transferred WT OT-I cells in skin-draining lymph nodes of recipient K14-mOVA mice (Figure 1k, 1l).

In conclusion, OT-I cell−transferred recipient K14-mOVA mice develop aGVHD-like mucocutaneous disease with histological evidences of LTR/IFD, depending on cytotoxicity mediated by PRF1 and GzmB, but not FasL, produced by OT-I cells. GzmA produced by OT-I cells presents partial cytotoxic activity compared to GzmB both in vivo and in vitro; this observation is supported by results of previous studies (Shi et al., 1992; Susanto et al., 2013). Reduced infiltration of PRF1⁻/⁻ OT-I cells and GzmB⁻/⁻ OT-I cells was also observed in the skin of K14-mOVA mice. CXCL9/10 mRNA expression in the epidermis of PRF1⁻/⁻ OT-I cell- and GzmB⁻/⁻ OT-I cell recipient K14-mOVA mice decreased compared to that in WT OT-I cell recipients, although activated PRF1⁻/⁻ and GzmB⁻/⁻ OT-I cells expressed CXCR3 as well as activated WT OT-I cells (Supplementary Figure S4 online). CXCL9/10 induces skin homing of activated OT-I cells in this murine model (Villarreal et al., 2014), and chemokine expression is reportedly up-regulated in apoptotic keratinocytes in inflammatory skin diseases (Klunker et al., 2003). Therefore, lack of keratinocyte apoptosis due to impairment of cytotoxic mechanisms in PRF1⁻/⁻ and GzmB⁻/⁻ OT-I cells in recipient mice may cause decreased release of CXCL9/10, resulting in mild infiltration of PRF1⁻/⁻ OT-I cells and GzmB⁻/⁻ OT-I cells into the skin.

Serpin3n has been reported as a murine biological extracellular protein with a serine protease inhibitory effect on the enzymatic activities of both murine and human GzmB (Haile et al., 2015; Sipione et al., 2006). To confirm the therapeutic effects of PRF1/GzmB pathway blockade in LTR/IFD, we treated OT-I cell−transferred recipient K14-mOVA mice with serpin3n in prophylactic and therapeutic administration protocols (Figure 2a). K14-mOVA mice treated prophylactically and therapeutically with serpin3n exhibited much milder eventual weight loss compared to the controls (Figure 2b). K14-mOVA mice treated prophylactically with serpin3n did not develop aGVHD-like disease until 14 days post−OT-I cell transfer (Figure 2c, 2d). Moreover, the aGVHD-like mucocutaneous lesions were remarkably improved in K14-mOVA mice that developed aGVHD-like mucocutaneous lesions 5 days post−OT-I cell transfer upon therapeutic serpin3n treatment (Figure 2c, 2d). Pathological findings revealed subtle LTR/IFD and decreased numbers of infiltrating OT-I cells (Figure 2e−2i).

Our results indicate that serpin3n administration has prophylactic effects on LTR/IFD disease development and remarkably improves mucocutaneous lesions after disease onset in vivo. We therefore suggest that serpin3n could be an attractive biological agent specific for the pathogenesis of skin disorders with LTR/IFD.

CONFLICTS OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Miwako Shobo for her technical support. This work was supported by grants from MEXT KAKENHI, TR-SPRINT, AMED, and the Canadian Institutes of Health Research (15K09757 and 16-26). This study was approved by the Institutional Animal Care and Use Committee in University of Tsukuba (17-135).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.03.1507.

REFERENCES


