density and diameter, were approximately twice as large in this study as those of normal skin in previous reports such as Liew et al. [6], in which the body sites studied were not the face. The difference in vessel parameters might be caused by the difference in body site or angiographic methodology, and further studies will be needed to distinguish between these possibilities.

In this pilot study, we demonstrated that OCT angiography can be used to evaluate alterations in facial vasculature with age. The evaluation of dermal vascular plexuses by means of OCT angiography has several advantages. It produces site- and depth-specific images with high-resolution. It is non-invasive and non-destructive. Skin specimens are known to shrink approximately 18% in width immediately after surgical excision [9]. Moreover, it has been suggested that blood vessel diameters measured in histological sections are smaller than those measured in OCT images *in vivo*, because of the dehydration of histological sections and collapse of the blood vessels' structure [10]. Therefore, information about vascular structure obtained by OCT angiography should more accurately reflect *in vivo* biological conditions, compared with conventional histological approaches. On the other hand, the time needed to acquire one angiographic image from time-sequential OCT tomograms is typically in the range of several minutes, because the algorithms used to reconstruct the image are based on the analysis of blood flow. Therefore, skin immobilization is an important consideration, and there are some skin regions that may be difficult to immobilize, for example regions around the nose and eye. Furthermore, it should be noted that OCT angiography reconstructs an image of blood vessels by extraction of moving regions, and so it is difficult to distinguish between veins, arteries and postcapillary venules.

In conclusion, our study demonstrates that OCT angiography is a useful technique to non-invasively address vascular alterations in facial skin. Further development of OCT angiography and the accumulation of *in vivo* data would be beneficial for the diagnosis of skin disease, as well as the validation of medical applications.

Conflict of interest disclosure

YH, TY, KK, CK and KK are employed by Shiseido Co., Ltd., which funded this project.

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Letter to the Editor

Requirement of MHC class I on radioresistant cells for granzyme B expression from CD8$^+$ T cells in murine contact hypersensitivity

To the editor,

Allergic contact dermatitis (ACD) is a common cutaneous disorder that affects around 15–20% of the general population worldwide. Haptens, chemical compounds of molecular weight smaller than 500 Da, are assumed to react with self-peptides or −proteins to serve as pathogenic antigens (Ags) in ACD [1,2]. Ag-presented-CD8$^+$ T cells at the elicitation phase produce interferon (IFN)-γ and cytotoxic granules such as granzyme B and perforin, and provoke inflammation [2–4]. Dermal dendritic cells (dDCs) are considered essential for the presentation of these haptenized-Ag to CD8$^+$ T cells [2,5,6]. On the other hand, given that haptens can modulate peptides that are attached to major histocompatibility complex (MHC) class I [1], and that MHC class I is expressed in a variety of cells other than dDCs, dDC-independent Ag-presentation by radioresistant cells has been assumed in ACD from some *in-vitro* studies [7–9], however, the actual contribution of which *in vivo* is poorly understood.

Abbreviations: ACD, allergic contact dermatitis; CHS, contact hypersensitivity; Ag, antigen; MHC, major histocompatibility complex; IFN-γ, interferon-gamma; β2m$^{-/-}$, beta-2-microglobulin-deficient.

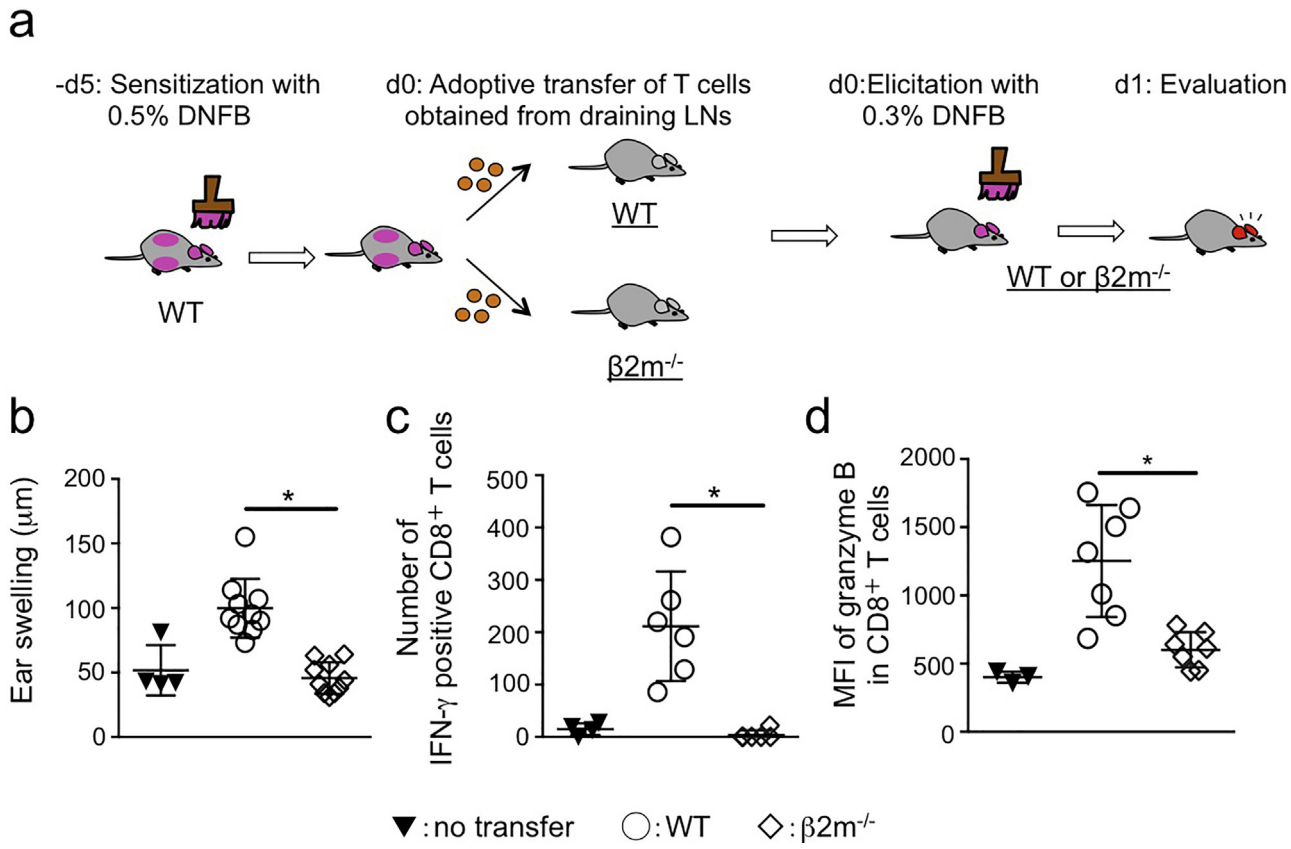

Fig. 1. CHS responses in WT or β2m$^{-/-}$ mice with adoptive transfer of effector T cells. a, A schema of CHS in WT or β2m$^{-/-}$ mice with adoptive transfer of T cells from sensitized mice. **b–d,** Ear swelling levels (**b**), the number of IFN-γ positive CD8$^+$ T cells (**c**), and MFI of granzyme B in CD8$^+$ T cells (**d**) in the ear skin of WT and β2m$^{-/-}$ mice transferred with T cells from 0.5% DNFB-sensitized WT mice and elicited with 0.3% DNFB. *, $P < 0.05$. WT mice without adoptive transfer of T cells and elicited with 0.3% DNFB were set as a negative control.

Herein, using a murine contact hypersensitivity (CHS), an animal model of ACD, we attempted to clarify the possibility of dDC-independent Ag-presentation at the elicitation.

We first confirmed the requirement of MHC class I on Ag-presentation in CHS, using β2-microglobulin-deficient (β2m$^{-/-}$) mice that lack MHC class I [10]. To focus on the elicitation phase, we performed an adoptive transfer experiment. T cells from 2,4-dinitro-1-fluorobenzene (DNFB)-sensitized wild-type (WT) mice were adoptively transferred to the recipient WT or β2m$^{-/-}$ mice, which were then applied DNFB to the ear skin for elicitation (Fig. 1**a**). In β2m$^{-/-}$ mice, the CHS response as estimated by ear swelling levels was significantly abrogated (Fig. 1**b**). In addition, production of IFN-γ and granzyme B in CD8$^+$ T cells was almost completely abolished in β2m$^{-/-}$ mice (Fig. 1**c and d**), indicating that MHC class I is actually essential for the elicitation.

Next, we tried to dissect the importance of MHC class I on radioresistant cells and radiosensitive cells using bone marrow chimeras. Bone marrow cells of WT mice or β2m$^{-/-}$ mice were donated into recipient WT mice (WT-to-WT and β2m$^{-/-}$-to-WT) or β2m$^{-/-}$ mice (WT-to-β2m$^{-/-}$) to establish each chimera. Then, CHS was induced in each chimera by adoptive transfer of T cells from DNFB-sensitized WT mice (Fig. 2**a**). In WT-to-β2m$^{-/-}$ chimeras, in which radioresistant cells such as Langerhans cells (LCs), keratinocytes, fibroblasts, blood endothelial cells, and lympatic endothelial cells lack MHC class I, which were confirmed by flow cytometry analysis (**Fig. S1**), the extent of ear swellings and IFN-γ production from CD8$^+$ T cells were comparable to those of WT-to-WT controls (Fig. 2**b and c**). The expression of granzyme B in CD8$^+$ T cells, however, was significantly attenuated in WT-to-β2m$^{-/-}$ chimeras (Fig. 2**d**). These results indicate that MHC class I on radioresistant cells is dispensable for IFN-γ production, but is required for the granzyme B expression in CD8$^+$ T cells.

We also checked the CHS responses in β2m$^{-/-}$-to-WT chimeras, in which radiosensitive cells including dDCs were expected to lack MHC class I, therefore, the chimera should exhibit impaired ear swelling responses and IFN-γ production [6]. dDCs in the β2m$^{-/-}$-to-WT chimera, however, exhibited marginal but significant expression of MHC class I compared to that of β2m$^{-/-}$ mice (**Fig. S2**). In addition, β2m$^{-/-}$-to-WT chimeras exhibited normal ear swelling responses and IFN-γ production (Fig. 2**b and c**), whereas the expression of granzyme B in CD8$^+$ T cells was significantly attenuated (Fig. 2**d**). These results indicate that, in β2m$^{-/-}$-to-WT chimeras, dDCs slightly expressed MHC class I, which induced IFN-γ production but was not sufficient to induce granzyme B expression in CD8$^+$ T cells.

Taken together, our data indicate that, in CHS, MHC class I on radioresistent cells mediates granzyme B expression in CD8$^+$ T cells, while MHC class I on radiosensitive cells mediate both IFN-γ and granzyme B production.

Although it remains unknown which radioresistant cells mediate the granzyme B expression from CD8$^+$ T cells in the skin, keratinocytes are one of the candidates considering their capacity to present haptens *in vitro* [7,8]. Given that infiltrative T cells are frequently observed close to blood vessels in CHS and ACD [6], and that there is a report that suggest the contribution of endothelial cells for Ag-presentation [9], blood endothelial cells might also be participated in Ag-presentation and granzyme B expression. LCs may be another candidate population, because LCs possess strong Ag-presentation ability *in vitro*. However, the contribution of LCs seems quite limited, because CHS responses including ear swelling