Infiltration and Clustering of Major Histocompatibility Complex II⁺ Antigen-Presenting Cells in the Skin of Patients with Atopic Dermatitis

TO THE EDITOR

Atopic dermatitis (AD) is one of the most common inflammatory skin diseases and affects up to 20% of the population in the western societies (Weidinger and Novak, 2016). AD is characterized by a complex genetic, environmental, and immunological factor—triggered skin barrier dysfunction, with mixed T helper cell responses (Brunner et al., 2018; Gittler et al., 2012). The pathophysiology of AD is tightly associated with the activation and interaction of multiple local skin immune cells, including different types of antigen-presenting cells (APCs) (Peng and Novak, 2015; Singh et al., 2016). However, the knowledge about the organization of skin immune cells in AD skin is still largely unclear.

Tissue inflammation can trigger the formation of tertiary lymphoid structures by the immune cells, which exert complementary functions along with the classic lymphoid tissues (Cabrita et al., 2020). Furthermore, inducible perivascular leukocyte clusters as sublymphatic structures exist in the skin of people with contact dermatitis (Natsuaki et al., 2014). Because all APCs express the major histocompatibility complex (MHC) II molecules (Kambayashi and Lauer, 2014), we stained the MHC II molecules to investigate the overall infiltration and organization of APCs in the skin of patients with AD. The study was approved by the ethics committee of University of Bonn, Germany. After written informed consent of the patients was obtained, skin specimens were taken. We first evaluated the expression of MHC II molecules in the skin of patients with AD and healthy subjects by performing immunofluorescence staining of skin sections. As compared with healthy skin, higher numbers of MHC II⁺ cells, as well as elevated MHC II expression, were observed in the skin of patients with AD (Figure 1a and b).

Dendritic cells (DCs) are the most important APCs orchestrating T-cell responses. The flow cytometric analysis of fresh AD skin biopsies demonstrated that the expression of MHC II on CD1a⁺ skin DCs in AD positively correlated with the expression of MHC I, activation marker CD83, and costimulatory molecules B7H3, CD80, and CD86, reflecting the activation of DCs in the skin of patients with AD. No correlation of MHC II expression on CD1a⁺ skin DCs with other surface markers such as langerin/CD207, membrane-bound IgE, and FcεRI (high-affinity receptor for IgE) and levels of total serum IgE of the patients with AD was observed (Figure 1c).

Enhanced apoptotic death of keratinocytes (KCs) leading to spongiosis exists in AD skin. Infiltrating T cells and T-cell–released inflammatory cytokines play essential roles in inducing KC apoptosis in the skin of patients with AD (Rebane et al., 2012; Zimmermann et al., 2011). In line with previous studies, the infiltration of MHC II⁺ APCs and T cells was detected in both the dermis and the epidermis of patients with AD (Supplementary Figure S1 and S2a). Interestingly, the presence of MHC II⁺ APCs, but not T cells, was also observed at the sites of KC spongiosis (Supplementary Figure S2b). Furthermore, we observed the migration of MHC II⁺ APCs between epidermis and dermis, and the MHC II⁺ cells were located close to the loosely connected KCs (Supplementary Figure S2b). The increased KC apoptosis, which contributes to the epidermal barrier dysfunction, has been demonstrated to be related to the secretion of IFN-γ (Rebane et al., 2012) and the coregulation of TWEAK and TNF-α (Zimmermann et al., 2011). Our results imply that both T cells and APCs might be the two major cell types that induce spongiotic KCs in the epidermis of patients with AD.

The skin infiltration of various types of immune cells is a hallmark of AD. Our triple immunofluorescence staining results demonstrated the existence of leukocyte clusters in the dermis of AD skin, in which the MHC II⁺ cells and T cells used the structure as the base to migrate in and out of the skin (Figure 2a and Supplementary Figure S3). In contrast, those enlarged leukocyte clusters were not observed in the healthy skin (Figure 2b). In the skin of patients with AD, the diameters of the leukocyte clusters were significantly more enlarged than that of the common peripheral vascular vessels in the healthy skin (Figure 2b). Furthermore, we scanned the sections of AD skin with a microscope to observe the three-dimensional structure of the leukocyte clusters. In AD skin, we detected a massive infiltration and accumulation of MHC II⁺ APCs, T cells, and mast cells in the leukocyte clusters, in which close contacts were frequently observed among the MHC II⁺ cells and/or T cells and/or mast cells (Figure 2c). The counterstaining of MHC II with lymphocyte common antigen CD45 and DAPI further confirmed the substantive infiltration of leukocytes together with MHC II⁺ APCs in the clusters (Figure 2d). In addition, four-color immunofluorescence staining of T-cell marker CD4 or CD8, MHC II, T-cell activation marker CD69, and DAPI demonstrated the infiltration of CD4⁺ and CD8⁺ T-cell subsets in the cluster. The expression of the CD69 on CD4⁺ or CD8⁺ T cells in
the cluster demonstrated the activation of T cells in the structure (Figure 2e).

As a next step, we demonstrated that CD14⁺ monocytes, CD68⁺ macrophages, Langerin⁺ DCs, and CD20⁺ B cells were recruited into the leukocyte clusters in the AD skin (Supplementary Figure S4).

Indeed, MHC II expression is not only crucial for antigen presentation at the initiation phase of the disease but also for the maintenance of the immune homeostasis, which is vital for the disease development. Increased expression of MHC II molecules and costimulatory molecules, as shown in our study, allows skin DCs to regulate the activation of resident T cells. Because the immune cells attach closely to each other in this structure, the leukocyte clusters in the skin of patients with AD may provide a structure for the cross-talking of skin APCs and T cells.
Figure 2. Infiltration of MHC II⁺ APCs, T cells, and mast cells in the perivascular leukocyte clusters of AD skin. The 4-mm cylindrical cores of skin punch biopsies were taken from healthy individuals (n = 5 donors) or patients with AD (n = 5 donors) as described elsewhere. (a) Paraffin-embedded AD skin sections were prepared and stained with antibodies against HLA-DR, HLA-DP, HLA-DQ (Alexa Fluor 594, red color) and CD3 (Alexa Fluor 488, green color). The coexistence of MHC II⁺ APCs and CD3⁺ T cells in the perivascular leukocyte clusters was shown. (b) Paraffin-embedded CTR and AD skin sections were stained with antibodies against HLA-DR, HLA-DP, HLA-DQ (Alexa Fluor 594, red color), CD3 (Alexa Fluor 488, green color), and tryptase (Alexa Fluor 405, blue color). Enlarged leukocyte clusters in the skin of patients with AD (left panel) and diameters of the leukocyte clusters (right panel) in the skin of healthy individuals and patients with AD were shown. (c) Skin sections were scanned by the microscope after three-color immunofluorescence staining for CD3 (Alexa Fluor 488, green color), MHC II (Alexa Fluor 594, red color), and tryptase (Alexa Fluor 405, blue color). A typical immunofluorescence picture (left panel) and 3D structure (middle panel: overhead view; right panel: side view) of the leukocyte cluster were shown. (d) Skin sections from the patients with AD (n = 5 donors) were stained with antibodies against HLA-DR, HLA-DP, HLA-DQ (Alexa Fluor 594, red color), CD45 (Alexa Fluor 488, green color), and counterstained DAPI (blue color). Representative fluorescent images were shown. (e) Skin sections from the patients with AD (n = 5 donors) were stained with antibodies against HLA-DR, HLA-DP, HLA-DQ (Alexa Fluor 594, red color), CD69 (Alexa Fluor 647, pink color), CD4 or CD8 (Alexa Fluor 488, green color), and DAPI (blue color). Representative fluorescent images were shown. White dashed line delineated the boundary between epidermis and dermis, whereas yellow dashed cycle line delineated the boundary of skin leukocyte cluster. Bar = 50 um. 3D, three-dimensional; AD, atopic dermatitis; APC, antigen-presenting cell; CTR, control; MHC, major histocompatibility complex.
Together, our results demonstrated that the skin immune cells, including MHC II⁺ APCs, T cells, and mast cells, are aggregated as leukocyte clusters, where cell interaction and communication might play fundamental roles in the development of AD. The detailed function of MHC II on skin APCs in regulating the skin immunity of AD needs to be investigated in the future. A better understanding of the organization of immune cells in the human skin is vital for the development of new treatments for AD. Our data also suggest that clinical drugs targeting the formation of the leukocyte clusters by suppressing the activation of APCs or T cells could be used to control the skin lesion and epidermal barrier dysfunction in patients with AD.

Supporting information
The materials and methods are described in the Supplementary Materials.

Data availability statement
No datasets were generated or analyzed during this study.

CONFLICT OF INTEREST
JO reports grants and/or personal fees from Bayer, Biotest, Chugai Pharmaceuticals, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum AB, Shire and/or Takeda Pharmaceutical Company outside the submitted work. JO received personal fees for travel support, participation in advisory boards and participating in symposia as chair or speaker. The remaining authors state no conflicts of interest.

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Wenning Peng1, Bartlomiej Kwick2, Chunfeng Yu1, Natalio Garbi1, Jean-Pierre Allam1, Johannes Oldenburg4 and Natalija Novak1,3*

1Department of Dermatology and Allergy, University of Bonn, Bonn, Germany; 2Department of Dermatology and Immunodermatology, Medical University of Warsaw, Warsaw, Poland; 3Institute of Experimental Immunology, University of Bonn, Bonn, Germany; and 4Institute of Experimental Hematology and Transfusion Medicine, University of Bonn, Bonn, Germany
*Corresponding author e-mail: Natalija.Novak@ukbonn.de

SUPPLEMENTARY MATERIAL
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REFERENCES

Targeting the Jak/Signal Transducer and Activator of Transcription 3 Pathway with Ruxolitinib in a Mouse Model of Recessive Dystrophic Epidermolysis Bullosa—Squamous Cell Carcinoma

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Abbreviations: cSCC, cutaneous squamous cell carcinoma; RDEB, recessive dystrophic epidermolysis bullosa; STAT, signal transducer and activator of transcription

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TO THE EDITOR
Early-onset highly metastatic cutaneous squamous cell carcinomas (cSCCs) occur in the majority of patients with recessive dystrophic epidermolysis bullosa (RDEB)