Letter to the Editor

Platelet-activating factor decreases skin keratinocyte tight junction barrier integrity

To the Editor:

Keratinocytes (KCs) build the epidermis, an efficient barrier that prevents microbial pathogens and environmental agents including allergens from entering into the skin.[1] Pathological changes in skin KCs are commonly associated with many skin diseases, such as atopic dermatitis (AD), urticaria, and psoriasis. It was recently reported in the *Journal of Allergy and Clinical Immunology* that there are tight junction (TJ) defects that lead to a disruption of epithelial barrier function of KCs in the skin of patients with AD[2] and in the meantime evidence for the genetic and immune regulatory mechanisms that control TJ defects has been reported.[1,3,4] Barrier-related molecules in the epidermis are regulated by inflammatory responses derived from both the epidermal (ie, KCs and Langerhans cells) and immune cells (ie, T cells, dendritic cells, and other cells). Platelet-activating factor (PAF) is an important proinflammatory factor produced and secreted by several types of cells, including mast cells, monocytes, tissue macrophages, platelets, eosinophils, endothelial cells, and neutrophils, and is involved in allergic inflammation.[5] In this letter, we emphasize the influence of PAF on skin barrier and KC TJ integrity in AD and further analyze TJ disruption in diseased skin for better understanding the molecular and cellular mechanisms in the regulation of TJ barrier integrity.

First, we investigated the role of PAF in the regulation of TJ function of human KCs. Normal human skin KCs from healthy subjects and skin KCs from patients with AD were cultured at air-liquid interface (ALI) and transepithelial resistance (TER) was measured as a readout for barrier integrity. Two days after reaching maximal TER, ALI cultures were stimulated with different concentrations of PAF and TER was measured before and 24, 48, 72, and 96 hours after stimulation. In resting conditions without any stimulation, TER levels in AD KCs were significantly lower than in healthy KCs (Fig 1, *A*). PAF decreased the TER starting from 48 hours and after stimulation of ALI cultures of human KCs. Although AD KCs seem to show a relatively late response to PAF compared with healthy KCs in the early time points such as 24 and 48 hours, there was no difference in percent increase after 72 and 96 hours (Fig 1, *B*). Notably, changes in TER are taking place relatively late, becoming significant after 48 hours. A part of this effect can be due to secondary mediators, such as cytokines that modify TJ integrity. It has been reported that PAF activates epithelial cells to release several cytokines and several cytokines have been reported to regulate TJs.[5] In accordance with this, we observed an increase in paracellular flux of fluorescein isothiocyanate–labeled dextran 4 kDa in response to PAF treatment, indicating a comparable decrease in TJ barrier integrity in both groups (Fig 1, *C*).

Upon binding to the PAF receptor, PAF activates downstream signaling pathways leading to increased intracellular Ca^{2+} flux.[6] To investigate the downstream regulation of PAF on TJ, we stimulated KCs of healthy individuals and KCs of individuals with AD with ionomycin, an agonist inducing intracellular Ca^{2+} flux, and found that ionomycin rapidly decreased TER (Fig 1, *D*). The present study strongly suggests that the influence of PAF on barrier function is controlled by the induction of intracellular Ca^{2+} influx, because induction of Ca^{2+} flux alone with Ca^{2+} ionophore in the epithelium disrupts TJ barrier integrity. This finding does not exclude other signaling events via the PAF receptor, and has implications on other molecules working on the same family of G-protein coupled receptors that are inducing Ca^{2+} flux. As repeatedly reported in other cells, we also observed that the leakiness of AD KCs stays stable after many passages. We agree that there might be many reasons for this finding including prolonged exposure to T_H2 environment starting early in life. It is practically not possible that these epigenetic changes are the same in every individual, so variation between donors can be observed.

To investigate TJ barrier directly in the context of human skin diseases, we analyzed mRNA expression and TJ protein integrity in skin biopsies. Immunofluorescence staining of occludin and claudin-7 showed an intact TJ layer in healthy skin (Fig 2, *A* and *B*). TJ integrity was disrupted in AD skin, more severely in lesional AD than in nonlesional AD skin, manifesting with a patchy, disrupted, and less dense arrangement of protein expression of TJs (Fig 2, *B*). A full disruption of TJs expression was observed in some parts of AD lesions. Skin prick tests induce immediate allergic reaction and urticaria is another PAF-associated skin disease. As an important finding that may open a new window for future research, the TJ layer integrity was decreased in skin prick tests and urticaria in comparison to healthy skin (Fig 2, *A*).

We then further analyzed whether the defects in protein expression of TJs were due to a transcriptional regulation that affects mRNA levels. We used a low-density array microfluidic card (Applied Biosystems, Carlsbad, Calif), which contains all known junctional and junction-associated proteins that are expressed in the epidermis and epithelia.[7] In addition to TJ proteins, desmosomes, gap junctions, adherens junctions, and adaptor protein genes were included in the analysis. We observed decreased mRNA expression of claudin-7 in skin prick test biopsies, and decreased mRNA expressions of claudin-3, claudin-7, claudin-8, claudin-14, and occludin in urticaria biopsies compared with healthy skin biopsies (see Fig E1 in this article's Online Repository at www.jacionline.org). Similarly, the mRNA expressions of occludin, claudin-7, claudin-2, claudin-8, claudin-12, claudin-23, as well as several other junctional proteins such as multi-PDZ domain protein-1, plakophillin-2, and connexin-43 were downregulated in both nonlesional and lesional AD skin. In accordance with the confocal staining data, the decrease in mRNAs expression was stronger in lesional AD than in nonlesional AD skin (see Fig E2 in this article's Online Repository at www.jacionline.org).

Epithelial barrier disturbance is now recognized as a common feature in many inflammatory diseases including food allergy, chronic rhinosinusitis, asthma, and AD.[8] In the study by De Benedetto et al,[9] the expressions of claudin-1 and claudin-23 were reduced in nonlesional AD skin, which was also verified in the present study. Silencing of claudin-1 led to a reduction in TER, suggesting that the reduced expression of claudin-1 in AD epidermis is an important factor disturbing barrier integrity. Here, we demonstrate that barrier mechanisms are more complex

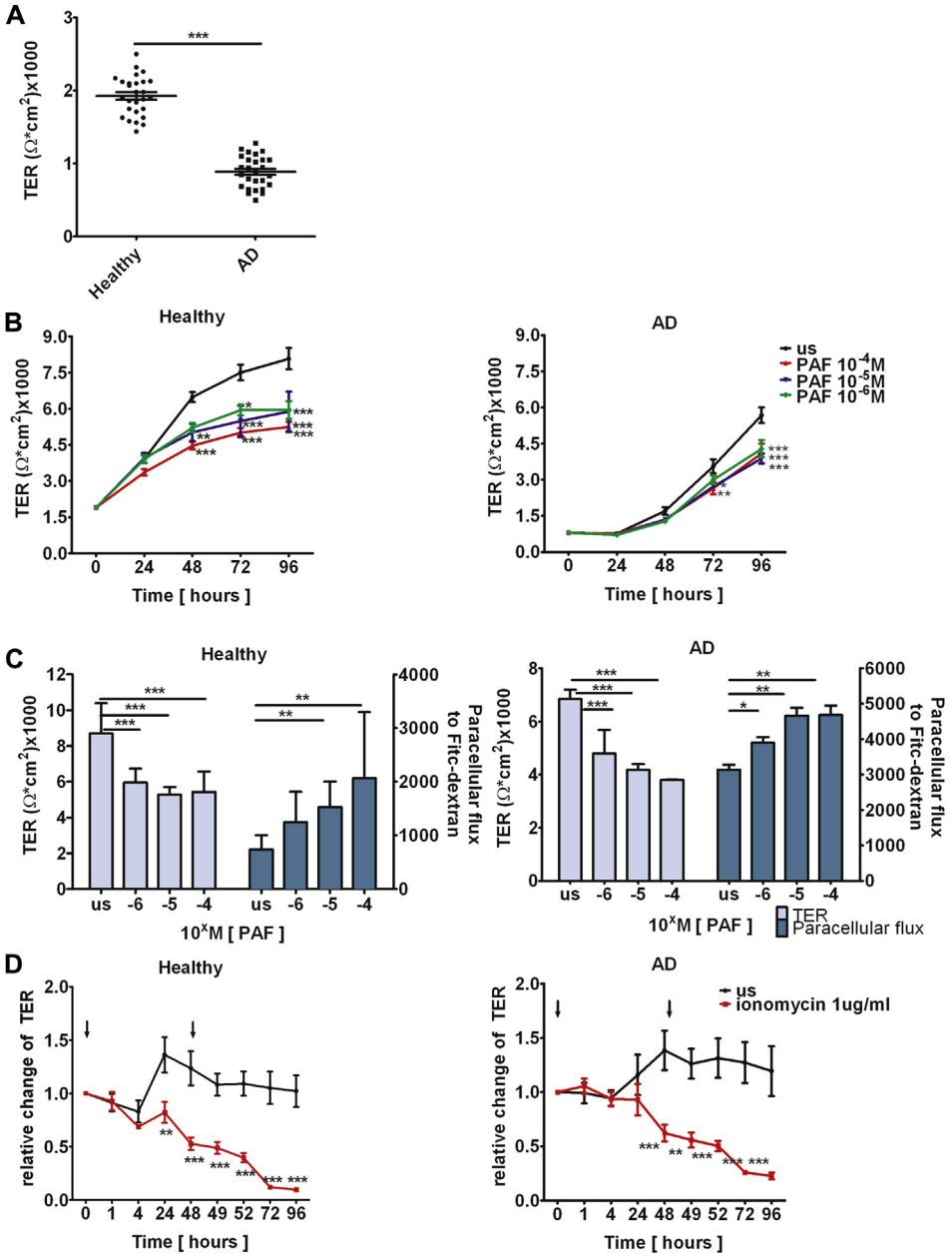

FIG 1. PAF reduces KC TJ barrier. **A,** Comparison of baseline TER in ALI cultures of human KCs from healthy subjects and patients with AD in resting conditions without any stimulation. ***$P < .001$ between 2 groups. **B,** Effect of PAF on TER in ALI cultures of human KCs from healthy subjects and patients with AD. **C,** Effect of PAF on FITC-dextran penetration of ALI cultures of human KCs from healthy subjects and patients with AD. **D,** Effect of ionomycin on TER in ALI cultures of human KCs from healthy subjects and patients with AD. The data represented duplicates in 3 independent donors. *$P < .05$, **$P < .01$, and ***$P < .001$ compared with 0 hour.

and other claudins are also involved in the reduced barrier of TJs in AD. The interaction of the skin barrier with inflammatory cells and mediators is also observed in essential stratum corneum barrier molecules, such as filaggrin. Skin KCs can release IL-33 under inflammatory conditions, and IL-33 disrupts the skin barrier by downregulating filaggrin.[4] Novel findings on the regulation of mucosal epithelial barrier by the immune system are continuously being reported during the last few years. Recently, it was demonstrated that CpG-DNA, which is recognized by Toll-like receptor 9, can enhance the barrier function of bronchial epithelial cells and rescue IL-13–induced barrier impairment by increasing the expression of TJs.[10] In conclusion, our study demonstrates that PAF induces skin TJ barrier defect, thus participating in the pathogenesis of barrier dysfunction in AD and other skin conditions and represents a potential target for prevention and treatment.

PAF is a potent phospholipid-derived mediator, the role of which has been implicated in both allergic and nonallergic