Functional expression of TRPV1 in human peripheral blood basophils and its regulation in atopic dermatitis

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LETTERS

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To the Editor,

Atopic dermatitis (AD) is a chronic and itchy inflammatory skin disease, which severely affects the quality of life of patients. So far, the mechanism of chronic itch in AD is not fully understood. Increasing evidence suggests that the transient receptor potential vanilloid 1 (TRPV1) channel is involved in neuro–immune interaction mechanisms regarding itch and inflammation.[1] TRPV1 is a nonselective cation channel that is directly activated by noxious stimuli. Extraneuronal expression of TRPV1 has been described in immune cells, including mast cells, eosinophils, and neutrophils.[2] To date, nothing is known about the presence and functional role of this channel in human basophils. As basophils are key effector cells in AD[3], we investigated whether basophils express TRPV1.

We isolated human basophils from peripheral blood and analyzed TRPV1 expression on the mRNA level (Figure 1A,C,D). Basophils exhibited higher TRPV1 mRNA expression than PBMCs (Figure 1D). To confirm the mRNA data, we examined TRPV1 protein expression through flow cytometry. We found that basophils express TRPV1 at the cell surface (Figure 1E,F) and intracellularly (Figure 1G). As previously reported, intracellularly located TRPV1 possesses various functions in cells. We examined the subcellular distribution in greater detail by double immunofluorescence analysis and revealed that TRPV1 is colocalized with the ER and Golgi network and weak with mitochondria (Figure 1H).

Next, we investigated the functional role of TRPV1 by measuring transient changes of intracellular calcium levels. Activation of the channel with capsaicin caused a decrease in calcium concentrations (Figure 1B,I). This drop could be explained by calcium shifting from the cytoplasm to intracellular stores. Remarkably, priming of basophils with IL-3 significantly enhanced calcium flux (Figure 1I). Functionally, activation of TRPV1 with capsaicin induced IL-6 release (Figure 1J and Figure S1) and apoptosis (Figure 1K) but not degranulation of basophils (Figure S2). The reduction of viable basophils might explain the beneficial effect of capsaicin, which is investigated in treating itch.[4]

As it has been demonstrated that TRPV1 is upregulated in the skin of AD[5] we compared TRPV1 expression between healthy and AD patients through flow cytometry. Interestingly, we found a significant difference in surface content (Figure 2A,C), whereas total protein levels, as determined by intracellular staining (Figure 2D), and mRNA levels (Figure 2E) were unchanged. This suggests that TRPV1 levels are mediated by trafficking and not through differential gene transcription. We assume that TRPV1 expression is also altered under in vivo conditions. We confirmed this in AD skin sections, where we observed that almost all basophils are TRPV1 positive and located near PGP9.5-positive nerves (Figure 2F,G; healthy skin Figure S3). Moreover, TRPV1-positive basophils express CD63 and are apoptotic (Figure S4).

Next, we investigated which factors modulate TRPV1 trafficking under pruritic conditions. Remarkably, stimulation with IL-3 and NGFβ significantly increased TRPV1 surface expression (Figure 2H) and modulated the subcellular distribution (Figure 2K). Our results confirm previous studies that described sensitization of TRPV1 by proinflammatory factors. Zhang et al. showed that NGF rapidly increases membrane expression of TRPV1 in HEK cells.[6] Furthermore, we demonstrated that alteration of channel distribution, directly modulates function, as illustrated by our calcium flux experiments. Finally, surface content is regulated by the inflammatory mediators IL-33 and TNF-α (Figure 2I), which are increased in AD, and extracellular acidification (Figure 2J).

In conclusion, TRPV1 is highly expressed in human basophils and upregulated in AD. IL-3, NGFβ, IL-33, TNF-α, and extracellular acidification cause potentiation of TRPV1 channel expression. Changes in subcellular distribution modulate basophil functions, as demonstrated by enhanced calcium flux. Additionally, activation of the TRPV1 channel induces IL-6 release and apoptosis. Further, TRPV1-positive basophils in AD skin express CD63 assuming histamine release. Our data highlight a pro-inflammatory and pruritic role of TRPV1 in basophil function, marking TRPV1 as a potential target for therapeutic approaches in itch and inflammation, for basophil-associated inflammatory skin diseases such as AD.

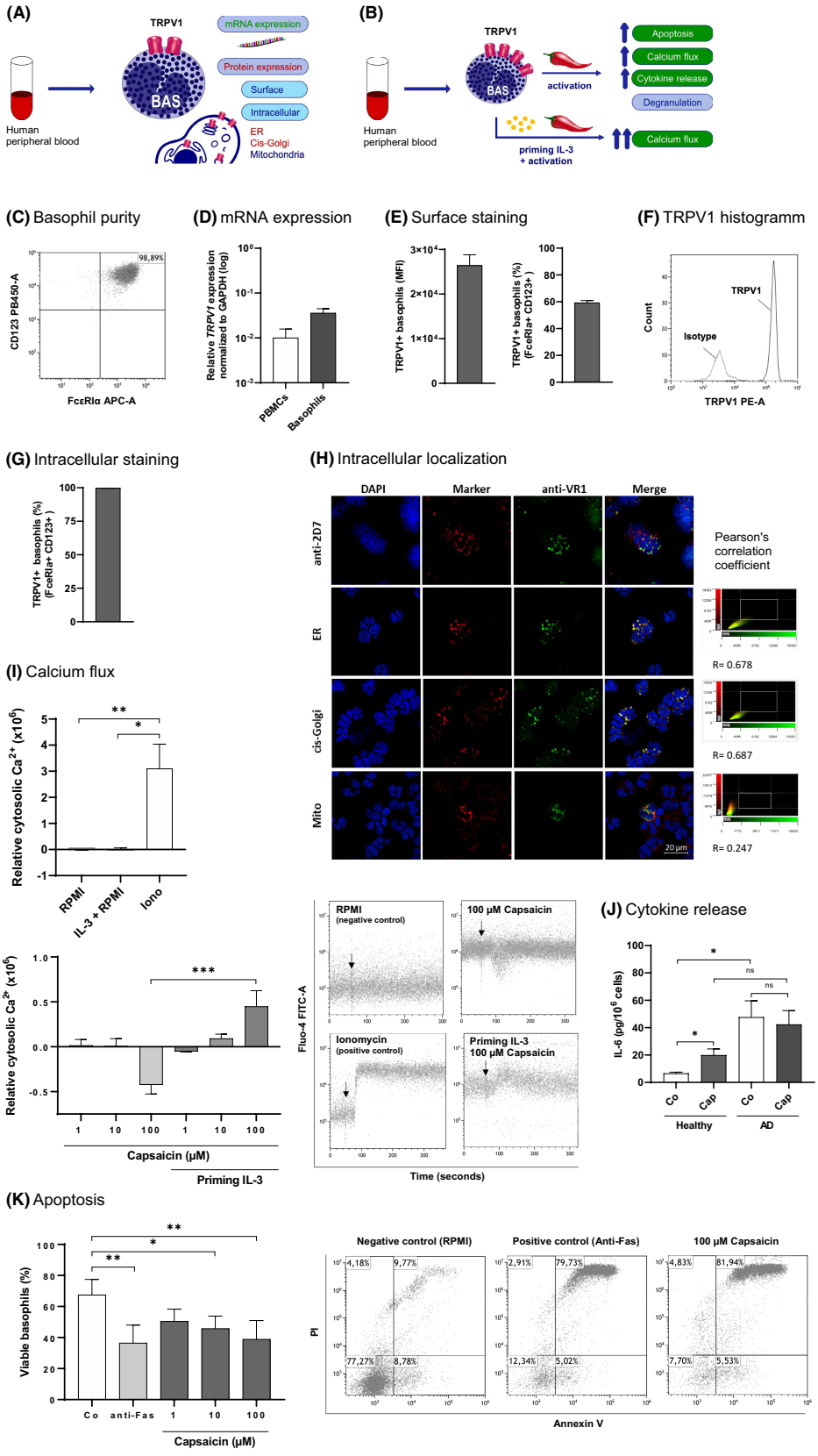

FIGURE 1 TRPV1 is functionally expressed in human basophils. Graphical summary of (A) TRPV1 expression and (B) function. (C) Purity of isolated basophils from peripheral blood by immunomagnetic cell sorting. (D) TRPV1 mRNA expression was determined by qRT-PCR ($n = 5$). (E) TRPV1 surface and (G) total protein expression (intracellular staining) were analyzed by flow cytometry ($n = 5$). (F) Isotype control. (H) Correlation and subcellular localization of TRPV1 (anti-VR1) in basophils (anti-2D7). (I) Calcium flux ($n = 4$) and (J) cytokine release ($n = 6$) after channel activation with capsaicin. (K) Apoptosis after activation with capsaicin ($n = 5$). SEM; $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

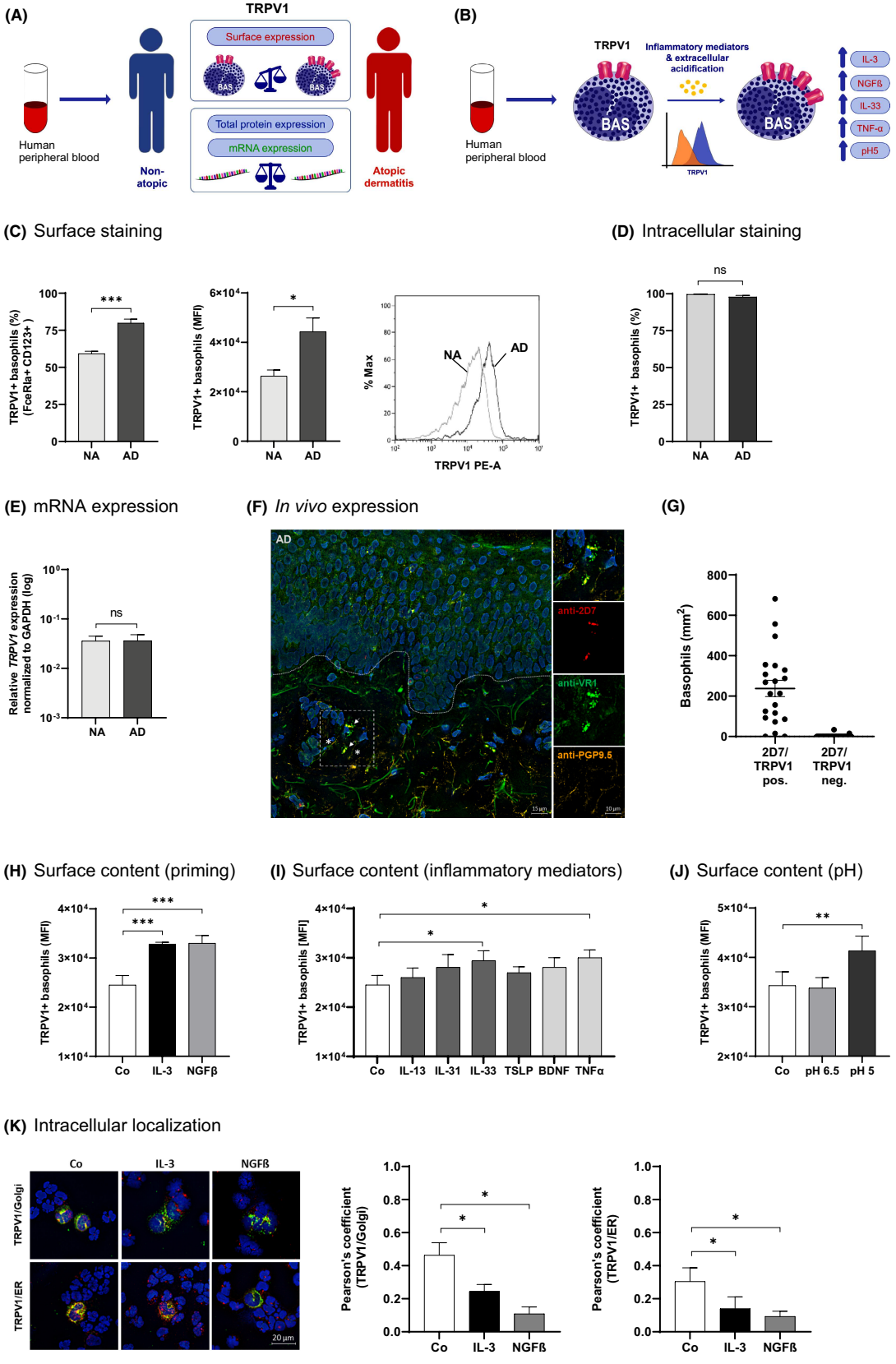

FIGURE 2 TRPV1 is upregulated in AD and modulated by inflammatory mediators. Graphical summary of (A) TRPV1 expression in AD and (B) modulation. (C) Comparison of TRPV1 surface, (D) total protein, and (E) mRNA expression (AD vs. nonatopic donors (NA); $n=6$). (F) In vivo expression of TRPV1 (anti-VR1) in basophils (anti-2D7) in AD-skin and localization to nerves (anti-PGP9.5) ($n=4$). (G) Quantification of TRPV1+ basophils. (H) Modulation of TRPV1 surface expression by basophil-activating factors (I) inflammatory mediators, and (J) extracellular acidification ($n=5$). (K) Modulation of subcellular distribution ($n=5$). SEM; $^{*}p<.05$; $^{**}p<.01$; $^{***}p<.001$. ns, not significant.

AUTHOR CONTRIBUTIONS

ML designed, performed, analyzed, and supervised experiments and wrote the manuscript. DW performed, analyzed, and discussed experiments. NG performed, analyzed, and discussed experiments. TW performed, analyzed, and discussed experiments. AB edited and revised the manuscript critically for important intellectual content. AK edited and revised the manuscript critically for important intellectual content. BH edited and revised the manuscript critically for important intellectual content. UR designed and supervised experiments, contributed to analysis interpretation, and edited the manuscript. All authors revised and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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