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LETTER

The cannabinoid WIN55212-2 suppresses effector T-cell responses and promotes regulatory T cells in human tonsils

To the Editor,

The human endocannabinoid system (ECS) is a complex molecular network encompassing cannabinoid receptors (CBRs), endocannabinoid ligands and the enzymes involved in their synthesis and degradation.[1,2] The role of cannabinoids in allergic diseases is not yet fully understood. Several studies reported that cannabinoids display beneficial anti-inflammatory properties, whereas others suggested potential proinflammatory and harmful effects.[2] The mRNA expression of functional cannabinoid receptor 1 (CB1) is upregulated in tonsils and peripheral blood of allergic patients.[3] Functional CB2 is also highly expressed in immune cells, and it is involved in the control of dendritic cell function.[2] We showed that the synthetic cannabinoid WIN55212-2 restores rhinovirus-induced epithelial barrier disruption[4] and promotes the generation of functional FOXP3$^+$ Treg cells in peripheral blood.[5] Blood dendritic cells (DCs) expressing CBRs are potential targets for cannabinoid-mediated modulation via autophagy and metabolic reprogramming.[5] Whether tonsil DCs preserve the ECS expression and how WIN55212-2 immunodulates T-cell responses in human tonsils remains unknown. Tonsils are first line secondary lymphoid organs located in the main entrance of the respiratory and gastrointestinal tracts where the generation of allergen-specific FOXP3$^+$ Treg cells occurs by mechanisms involving plasmacytoid DCs (pDCs).[6] Type 2 conventional DCs (cDC2s), which can be also found in the tonsil T-cell area, play an important role in the control of allergic diseases.[6] In the present study, we sought to investigate the protein expression pattern of CB1 and CB2 in tonsil DCs and whether WIN55212-2 could immunomodulate T-cell responses in human tonsils.

To study whether tonsil DC subsets express the ECS, we purified tonsil pDCs and type 2 conventional DCs (cDC2s) and quantified the in vivo mRNA expression levels of main ECS components (Figure 1A). Purified pDCs and cDC2s expressed CB1 and CB2, as well as the enzymes involved in the degradation of the endocannabinoids (anandamide and 2-arachidonoylglycerol), fatty acid amide hydrolase (FAAH), and monacylglycerol lipase (MAGL) at mRNA level, indicating that tonsil DCs express in vivo main components of the ECS. To further explore the expression pattern of CBRs in human tonsil DCs, we analyzed CB1 and CB2 protein expression in pDCs and cDC2s by confocal microscopy. Human tonsil sections were stained with the validated HU210-Alexa488 probe for CB1 identification or with anti-CB2 polyclonal antibody in combination with anti-human CD123 (pDCs) or CD1c (cDC2s) antibodies. pDCs and cDC2s located at the T-cell area of human palatine tonsils expressed CB1 at the protein level (Figure 1B). Although CB2 mRNA levels were detected in pDCs and cDC2s, only cDC2s, but not pDCs expressed CB2 at the protein level in tonsils (Figure 1B), which is in accordance with our previous findings for circulating blood DCs subsets.[5] Interestingly, the synthetic cannabinoid WIN55212-2 significantly reduced cytokine production induced by TLR stimulation on purified tonsil pDCs and cDC2s, demonstrating the functionality of these receptors (Figure S1A,B).

Next, we wanted to investigate how the synthetic cannabinoid WIN55212-2 affects T-cell responses in human tonsils. We stimulated tonsil mononuclear cells (TMC) with anti-CD3/CD2/CD28 antibodies for 3 days to induce T-cell proliferation in the presence of different doses of WIN55212-2 and assessed T-cell proliferation by measuring ^{3}H-thymidine incorporation. WIN55212-2 decreased the proliferation of tonsil T cells in a dose-dependent manner (Figure 2A) without affecting cell viability (Figure 2B). After 3 days, WIN55212-2 significantly impaired the production of IFNγ, IL-5, IL-13, and IL-17 in activated TMC both at the mRNA and protein levels (Figure 2B,C). Remarkably, WIN55212-2 significantly increased the percentage of CD25$^+$CD127$^-$FOXP3$^+$ Treg cells in activated TMC (Figure 2E), without increasing IL-10 levels. These data are indicating that WIN55212-2 is able to suppress the activation of tonsil Th1, Th2, and Th17 effector cells while promoting the expansion or the novo generation of Treg cells.

Tonsils are the only easily accessible secondary lymphoid organs in humans. Our findings showing that tonsil pDCs and cDC2s express functional CBRs and that WIN55212-2 suppresses effector T-cell responses while enhancing FOXP3$^+$ Treg cells might well open new avenues for the development of future novel vaccines targeting DCs in human tonsils for the prevention and treatment of allergy and other immune-mediated diseases.

KEYWORDS

cannabinoids, effector T cells, regulatory T cells, tonsils

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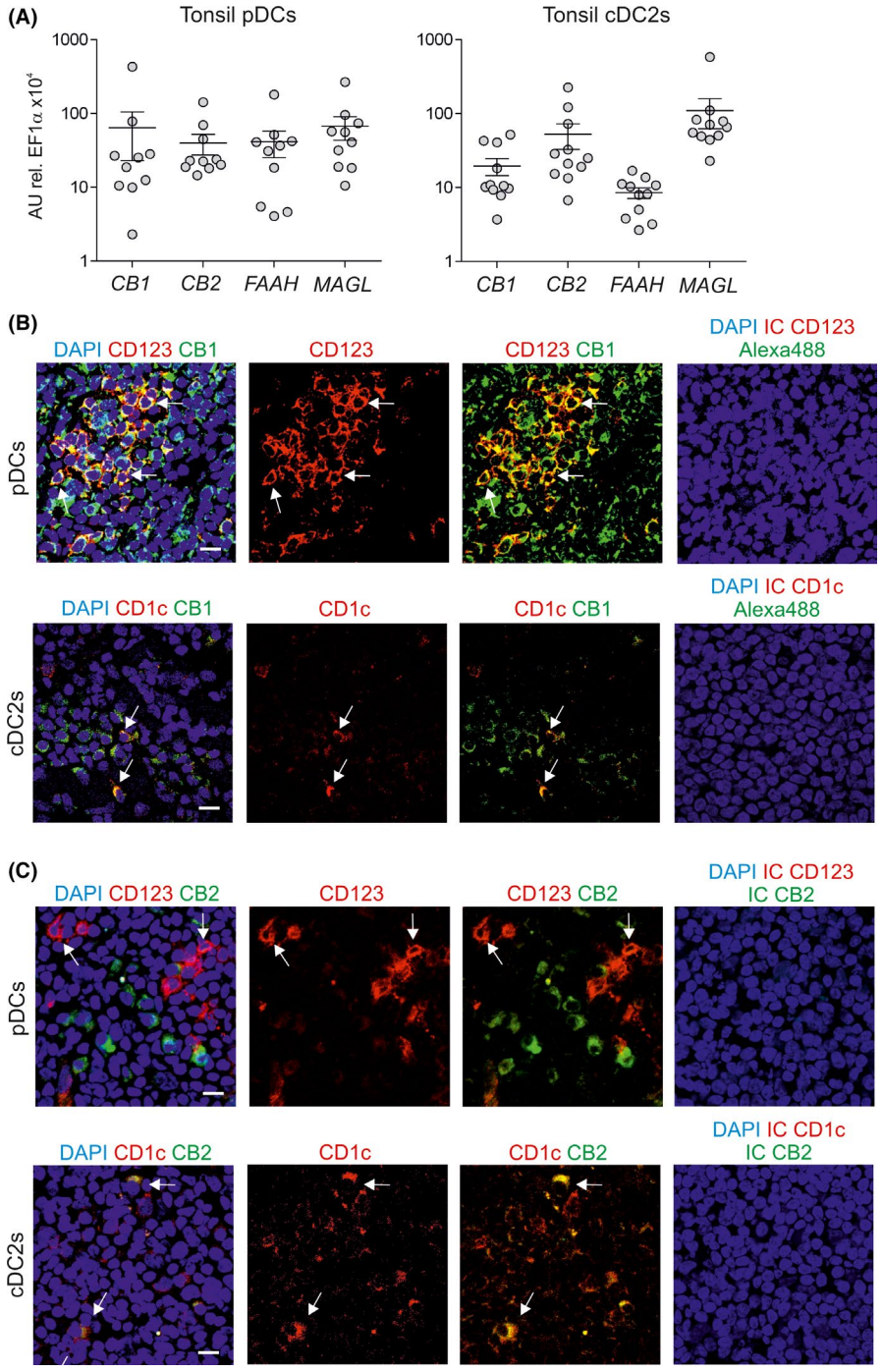

FIGURE 1 Tonsil DCs subsets express the main components of the ECS at mRNA level and CB1/CB2 at the protein level. (A) mRNA expression levels of CB1, CB2, FAAH, and MAGL in purified tonsil pDCs and cDC2. Arbitrary units (A.U.) are $2^{-(\Delta Ct)}$ values multiplied by 10^4, with ΔCt defined as the difference between the cycle threshold value for each gene and elongation factor 1 α (EF1α) as housekeeping gene. Visualization of (B) CB1 and C, CB2 expression in cDC2 and pDCs from human tonsils. Human tonsil sections were stained for CB1 or CB2 (green), for CD1c or CD123 (red) and with DAPI (blue, nuclei) and analyzed by confocal microscopy. White bars, 10 μm. White arrows point at cDC2 or pDCs in the T-cell area of the tonsil tissue. One representative example of three independent experiments with similar results. DAPI, 4′-6-Diamidino-2-phenylindole, dihydrochloride

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CONFLICT OF INTEREST

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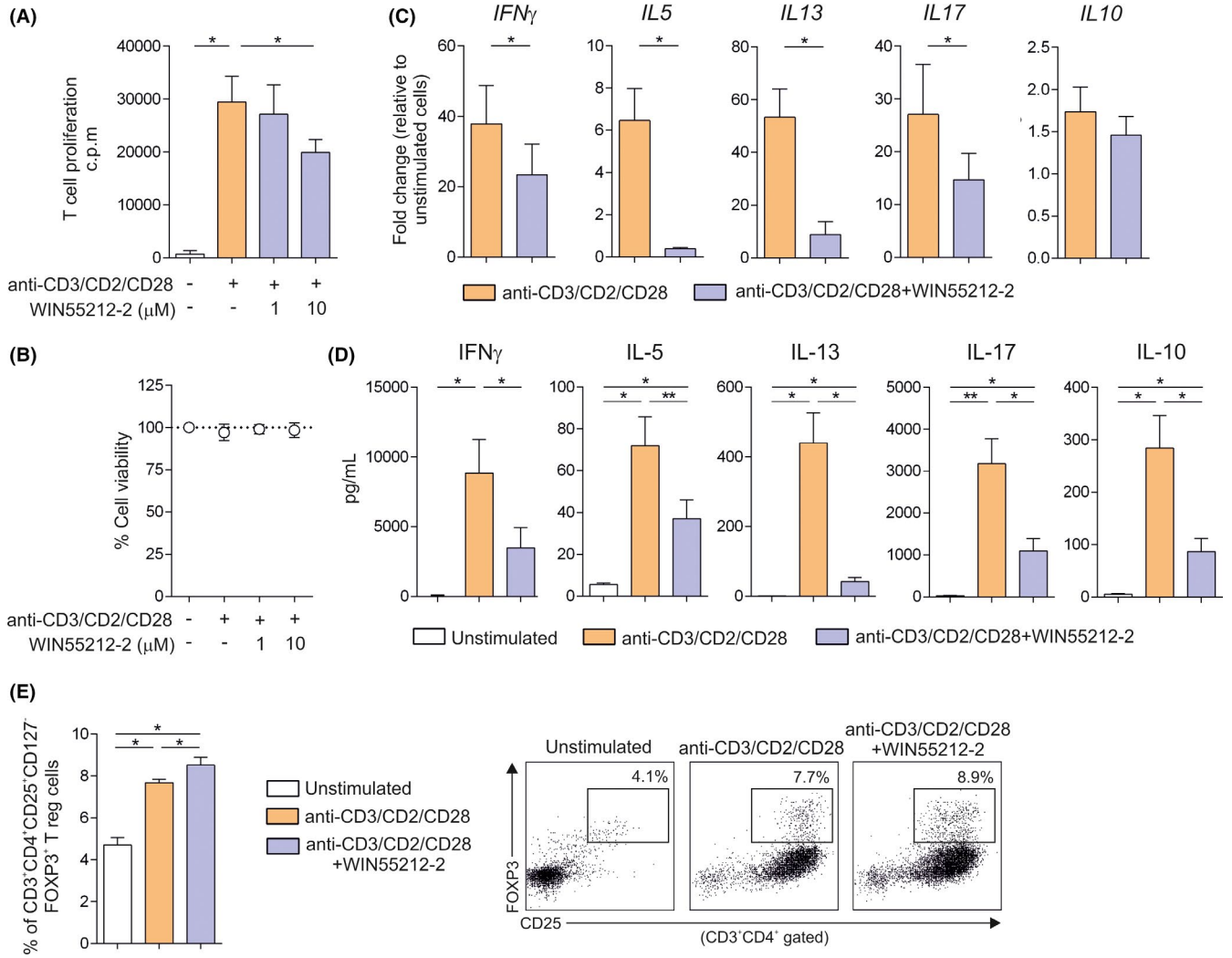

FIGURE 2 WIN55212-2 suppress effector T-cell responses and promotes Treg cells in human tonsils. (A) Freshly isolated TMC ($n = 5$ independent experiments) were cultured in medium or in the presence of the indicated stimulus: anti-CD2/CD3/CD28 alone to induce T-cell proliferation or in the presence of the indicated doses of the synthetic cannabinoid WIN55212-2 for 3 days. Proliferative responses were measured by using ^{3}H-thymidine incorporation as counts per minute (c.p.m.). (B) Cell viability at the indicated conditions. (C) mRNA expression levels of the indicated T cells cytokines in TMC stimulated for 3 days under the indicated conditions ($n = 5$ independent experiments) as determined by quantitative real-time RT-PCR. Fold changes with respect to the unstimulated conditions were calculated as $2^{-(\Delta\Delta Ct)}$, with ΔCt defined as the difference between the cycle threshold value for the gene of interest and elongation factor 1α (EF1α) as a housekeeping gene. (D) The levels of the indicated T-cell cytokines in cell-free supernatants ($n = 5$ independent experiments) were also quantified after 3 days by cytometric bead array. (E) Percentage of FOXP3^{+} Treg cells in TMC after 3 days of stimulation under the indicated conditions ($n = 4$ independent experiments). Flow cytometry representative dot plots are shown. *$P < .05$; **$P < .01$. Data represent the mean with SEMs

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