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Identification of the signaling pathway mediating the anti-inflammatory effect of cannabidiol on human sebocytes

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We have previously shown that endocannabinoids (e.g. anandamide, AEA) play important roles in the maintenance of the basal lipid synthesis of the human sebocytes. In contrast, (-)-cannabidiol (CBD), the major non-psychotropic phytocannabinoid of Cannabis sativa, was found to suppress unwanted sebaceous lipogenesis and exerted anti-proliferative and anti-inflammatory action. We have also described that the lipostatic and growth-inhibitory effects of CBD are mediated by the activation of transient receptor potential vanilloid 4 (TRPV4) channels; however, the molecular mechanism of its anti-inflammatory action is still unknown. Since the anti-inflammatory effect of CBD in a mouse lung injury model was dependent on the G_s-protein-coupled A2a adenosine receptor, in our current study, we first analyzed the expression of A2a on human SZ95 sebocytes. Using quantitative "real-time" PCR (Q-PCR), immuncytochemistry and Western blot, A2a receptors were identified on sebocytes. Moreover, CBD treatment elevated the intracellular cAMP concentration suggesting the activation of the receptor. We also demonstrated that CBD increased tribbles homolog-3 (TRIB3) expression, a negative regulator of the NF-κB pathway, the upregulation of which can be induced by the increased intracellular cAMP level. In addition, a specific A2a receptor antagonist (ZM241385) was able to prevent the up-regulation of TRIB3 by CBD. Of further importance, ZM241385 also suppressed the CBD-evoked inhibition of bacterial lipopolysaccharide-induced NF-κB-activation. Our results collectively suggest that CBD exerts its anti-inflammatory effect by the activation of "A2a receptor → cAMP → TRIB3 NF-κB" axis.

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Investigation of the endocannabinoid system on human monocyte-derived Langerhans cells

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Regulation of skin immune functions is key event in the maintenance of the cutaneous barrier. Langerhans cells (LC) represent the key professional antigen-presenting cells in the healthy epidermis, where they take part in the induction of both the immune response upon challenge from various infections and the tolerance towards autologous antigens in the absence of activating factors. The elucidation of regulatory mechanisms that influence LC functions represents an intensively studied field. One of the possible regulatory mechanisms is the endocannabinoid system (ECS), which has been shown to have profound effects on cutaneous immune functions. Research into the physiological processes of LCs is challenging since the isolation of these cells from the epidermis usually results in their activation while yielding relatively few cells. However, recent studies have reported that monocytes isolated from peripheral blood may be differentiated into LCs by treatment with a cytokine cocktail (IL4, GM-CSF and TGFβ1) for three days, followed by co-culturing of these LC-like cells with keratinocytes. Based on the model described above we differentiated human monocytes into LCs, and aimed at determining whether the cells express members of the ECS, and if endocannabinoids such as anandamide and 2-arachidonoyl glycerol (2-AG) affect the biological functions of these cells. Using quantitative real-time PCR we determined that these cells express numerous metabotropic (GPR18, GPR55 and GPR119) and ionotropic receptors (TRPV1, TRPV2 and TRPV4) linked to the ECS. Although anandamide and 2-AG had no measurable effect on the differentiation of monocytes into LCs, the cells themselves express relatively high levels of ECS-metabolizing enzymes (NAPE-PLD, MAGL, DAGLA, DAGLB, FAAH) that take part in the synthesis and degradation of endocannabinoid ligands. In summary, assessment of the ECS on LC promises to further increase our knowledge on the important immune-modulatory role of these cells in human skin physiology.

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Withdrawn

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Propionibacterium acnes activates the NLRP3 inflammasome in human SZ95 sebocytes

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Propionibacterium acne (P. acnes) and sebaceous glands are considered to play an important role in the development of acne. However, information regarding the activation of innate immunity by P. acnes in the sebaceous gland is limited. In this study, we investigated whether P. acnes activates the inflammasome in human sebaceous glands in vivo and in vitro. We found that IL-1β expression was upregulated in sebaceous glands of acne lesions. After stimulation of human SZ95 sebocytes with P. acnes, the activation of caspase-1 and secretion of IL-1β were enhanced significantly. Moreover, knocking down the expression of NLRP3 (but not AIM2) abolished P. acnes-induced IL-1β production in SZ95 sebocytes. The activation of the NLRP3 inflammasome by P. acnes was dependent on protease activity and ROS generation. Finally, we found that NALP3-deficient mice display an impaired inflammatory response to P. acnes. These results suggest that human sebocytes are important immunocompetent cells that induce the NLRP3 inflammasome, and that P. acnes-induced IL-1β activation in sebaceous glands plays an important role in acne pathogenesis.

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***Propionibacterium acnes* affects the cellular properties of cultured human keratinocytes in a strain-specific and dose-dependent manner**

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Propionibacterium acnes (*P. acnes*) bacterium is a member of the skin microflora, but may also serve as an opportunistic pathogen contributing to the pathogenesis of different skin diseases. Earlier we have shown that various *P. acnes* strains (*889*, *6609*, *ATCC 11828*) belonging to different phylogroups within the species differentially affected the proliferation and viability of cultured immortalized human keratinocytes (HPV-KER). We found that apart from this strain specificity, the extent of the induced cell biological changes greatly depended on the dose of the bacterial treatment; high doses of the pathogenic *889* and *ATCC 11828* strains resulted the death of the HPV-KER cells. In order to analyze this effect in more detail, we performed a fluorescent microscopic analysis of the *P. acnes* treated cultures, and found cells exhibiting altered morphology and extensive membrane blebbing, characteristic of membrane damage. This was also demonstrated by measuring the quantity of free lactate-dehydrogenase enzyme released to the supernatant from the damaged, bacterial-treated HPV-KER cells using an LDH assay. To demonstrate that the *P. acnes* induced cell damage is not a keratinocyte specific effect, we also treated human erythrocytes and quantified the rate of membrane damage by measuring the amount of free hemoglobin in the supernatant of the treated cells by spectrophotometry. We found, that in response to the high dose treatment of the same pathogenic strains (*889* and *ATCC 11828)* the amount of free hemoglobin increased. The same treatment conditions also caused marked pH changes (acidification) of the culture supernatants. These results suggest that *P. acnes* modifies the proliferation and viability of cultured HPV-KER cells in a strain-specific and dose-dependent manner, and this effect may be dependent on the production of an acidic factor possibly generated by the bacterium.

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***Pseudomonas aeruginosa* evades immune responses by mimicking the endogenous formation of host defence peptides from thrombin**

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen notoriously persistent in chronic infective conditions such as non-healing ulcers. This species is known for its immune evasive abilities amongst others by degradation of a large variety of host proteins. However, it has never been investigated whether protein degradation by *P. aeruginosa* enzymes may lead to the direct formation of bioactive peptides exerting novel functions. Therefore, the aim of this study was to investigate whether *P. aeruginosa* can generate peptides that modulate host responses. To study this concept we investigated thrombin degradation. Using a broad range of methods we found that *P. aeruginosa* elastase cleaves a C-terminal derived peptide from thrombin, which inhibits pro-inflammatory responses to several pathogen-associated molecular patters *in vitro* and *in vivo*. FACS, slot blot assays and electron microscopy showed that the peptide prevents receptor dimerization and subsequent activation of down-stream signalling pathways. Finally, small thrombin-derived C-terminal peptides were found in chronic wound fluids and on leukocytes derived therefrom. Taken together, *P. aeruginosa* elastase cleaves thrombin, resulting in the formation of a peptide that dampens inflammation. These findings constitute a novel concept of pathogen-host interactions, where bacteria mimic an endogenous anti-inflammatory mechanism that can aid in circumvention of host responses.