Effects of constant light exposure on the immune tolerance development in mice

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Circadian rhythm is an innate physiological rhythm which has been indicated to affect many aspects of the physiology and pathology including immune system and immunological diseases. Immune tolerance development is deeply related to the onset of inflammatory disorders. However, the effect of circadian rhythm in the mechanisms of immune tolerance development has not yet been fully clarified. Here we assessed the effects of circadian rhythm disruption on the immune tolerance induction by the perturbation of light environment, using a mouse model of neonatally induced cutaneous tolerance. Mice were kept under constant light (LL) or light-dark (LD) conditions, and hapten was applied at 2 days after birth for tolerance induction. Six weeks later, hapten was applied again for sensitization, followed by hapten application to ear skin 5 days later. The ear-swelling responses and cell infiltration into inflamed skin notably increased in LL mice compared with those in LD mice. Furthermore, the percentage ofFoxp3+ regulatory T cells markedly decreased in inflamed skin and draining lymph nodes of LL mice compared with that in LD mice. Loss-of-function mutation of a key circadian gene, Bmal1, also exacerbated the ear-swelling responses and cell infiltration accompanied by the decrease of Foxp3+ regulatory T cells into inflamed skin in mice. Taken together, circadian rhythms may be involved in the immune tolerance induction in allergic inflammation.

Induction of psoriatic dermatitis in mice following cutaneous purineergic P2X7 receptor signaling is dependent on neutrophil and inflammasome activation

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Psoriasis vulgaris is a cell mediated inflammatory cutaneous disease that affects approximately 2% of the US population. This disease is dependent on IL-17-secretting innate inflammatory cells and Th17 cells that are induced by cutaneous DCs and likely keratinocytes. Several triggers have been proposed as stimuli that induce DCs and keratinocytes to secrete IL-17-polarizing cytokines to generate the psoriasis phenotype. One potential stimulus is alarmins, such as ATP. ATP is a particularly interesting alarmin that, via purineergic P2X7 receptor (P2X7R) signaling, induces NF-κB activation and the IL-23/IL-17 axis, both of which have been shown to be psoriasis susceptibility pathways. We hypothesize that alarmins, such as ATP, play a pivotal role in the induction and maintenance of innate and adaptive cutaneous inflammation. Psoriasis is characterized by reporting that signaling through the P2X7R, in the presence of ATPaese inhibition, induces psoriasis dermatitis in mice characterized by uniform acanthosis, increased and dilated papillary dermal vascularity, parakeratosis, microabscess formation, and increased inflammatory cell infiltrates of macrophages, inflammatory monocytes and DCs, and neutrophils. Furthermore, additional characteristics of psoriasis were also present such as hair follicle miniaturization and diminished granular layer. Significantly increased innate cytokines, such as IL-1α, IL-β, IL-23, ST100α, and IL-6 were also observed. Furthermore, the inflammatory response induced following P2X7R stimulation is largely dependent on neutrophils and the IL-1β/IL1R1 inflammasome pathway. In conclusion, our results demonstrate that cutaneous inflammatory responses induced via purineergic signaling through the P2X7R have implications in the pathogenesis and potential treatment of inflammatory diseases, such as psoriasis.

Increased expression of stress hormone CRH receptor on circulating monocytes of alopecia areata patients

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Stress is believed to play a key role in alopecia areata (AA), although the exact interactions of stress with AA remain undefined. Corticotropin-releasing hormone (CRH), the proximal regulator of stress axis, has been recognized as an immunomodulatory factor in peripheral tissues and human peripheral blood mononuclear cells (PBMCs). We used multicolor flow cytometry to identify receptor CRHR1 expression on PBMCs subsets in AA patients (n=54) and controls (n=65). Then we performed in vitro CRH treatment on PBMCs to assess the response of the cells. We found that CRHR1 was expressed primarily by circulating monocytes, CRHR1 expression on monocytes was enhanced in AA compared with controls (3.17% versus 1.44%, p=0.011). High CRHR1 expression was significantly correlated to chronic AA (disease duration >1year, p=0.001, χ2 test), and large lesion area (AA >25%, p=0.027, χ2 test). High CRHR1 expression was also correlated to a low expression of PD-L1, and markedly independently correlated with AA incidence (R=0.282, p=0.022). In vitro CRH treatment of control PBMCs slightly promoted innate immune response related gene upregulation, but downregulated pathological inflammatory response genes. Nevertheless PBMCs from AA patients were largely insensitive to CRH treatment. However CRH significantly increased Lymphotoxin beta (LTB) gene expression in PBMCs of AA whereas there was no change in control PBMCs. Our data suggest that in CRH sensitive subjects, CRH could play a role in modulating inflammation during the disease. Furthermore, our data suggests that CRH may exacerbate autoimmune inflammation by promoting monocyte migration and LTB production.

Honokiol induced DNA demethylation in UV exposed mouse skin prevents suppression of immune sensitivity through functional reactivation of dendritic cells

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Solar ultraviolet (UV) radiation induced immunosuppression has been implicated in skin cancer risk; therefore the treatment options which can inhibit UVB-induced immunosuppression may be useful in the management of skin cancers. Topical treatment of honokiol, a phytochemical from Magnolia plant, inhibits UVB induced suppression of contact hyper-sensitivity (CHS) response in mice. Here, we report that inhibition of UVB-induced suppression of CHS in C3H/HeN mice by treatment of honokiol (0.5 and 1.0 mg/cm² skin area) significantly increased CHS responses. The treatment of UVB-exposed donor mouse inhibits the suppression of the CHS response in naive mice, while the recipient mice, obtained DNA-methylated DCs from non-honokiol treated but UVB-exposed donor mice showed significant suppression in the CHS response to UVB, which were not observed in mice treated with non-honokiol treated UVB-exposed donor mice. These data suggest that correction of DNA hypermethylation by honokiol may have a role in stimulation of immune system in UVB-exposed animals.

Large-scale screening for T cell epitopes in human alopecia areata

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Alopecia areata (AA) is an autoimmune form of hair loss. T cells are known to be both necessary and sufficient to cause the disease, and the clustering of both CD4+ and CD8+ T cells around the hair follicles (HFs) suggests local recognition of both HLA-A and HLA-B presented autoantigens. Since growing (anagen) HFs are preferentially attacked in AA, the prevailing dogma in AA is that epitopes derived from anagen-specific proteins are targeted by T cells. Here, we present a large-scale, unbiased screening approach for HLA-A1 and II epitopes. We derived a list of 313 HLA-expressed proteins from our gene expression and proteomic data and publicly-available sources, with a specific emphasis on anagen stage proteins. We next employed the currently described HLA-II epitope prediction and standard HLA A-specific prediction for common HLA-A variants to design a panel of overlapping peptides restricted to HLA-A*0201 and HLA-DR. The latter was chosen based on our GWAS analysis finding of a strong region of association within HLA-DR (p<0.01×10^-7) that is primarily from HLA-DRB1. The resulting peptide library is being screened for recognition by AA patient and control CD4 and CD8 peripheral blood T cells using IFNg ELISPOT analysis. Epitopes within candidate peptides will be further fine mapped, and tested for recognition by skin-infiltrating T cells. Knowledge of AA-associated antigens will aid the development of diagnostic tools and potentially enable future antigen-specific therapeutic strategies.

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Regulatory mechanisms of PD-1/PD-L1 pathway on CD8+ T cell activation in murine contact hypersensitivity

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The programmed cell death ligand 1 (PD-L1) pathway is one of the critical systems to regulate T cell activation, which has been extensively studied in chronic inflammatory conditions, including cancers, viral infections and autoimmune diseases. In acute inflammation such as contact dermatitis, however, whether and how this pathway contributes to the regulation of the inflammation remains unclear. To address this issue, we applied PD-L1 deficient mice to murine contact hypersensitivity (CHS). PD-L1-deficient mice exhibited significantly exacerbated CHS responses with enhanced IFN-γ production from CD8+ T cells and marked upregulation of the notable contribution of PD-1/PD-L1 pathway in CHS. In addition, administration of PD-L1 blocking antibody during the elicitation phase induced increased IFN-γ production from CD8+ T cells in the skin and prolonged ear swelling duration. These results suggest that PD-1/PD-L1 pathway modulates CD8+ T cell activation in the skin. To examine the responsible cell populations that mediate this pathway, we investigated the expression of PD-L1 by flow cytometry on candidate cells in the skin including Langerhans cells, Langerin+ dendritic cells (dDCs), Langerin−dDCs and keratinocytes, all of which were supposed to contribute to the regulation of CD8+ T cell activation in the skin. Although PD-L1 was expressed on all the cell populations in both the steady and inflammatory states during the elicitation, only Langerin+ dDCs upregulated the PD-L1 expression during the elicitation. During the priming phase, high PD-L1+ expression was observed on the skin-infiltrating T cells. Knowledge of AA-associated antigens will aid the development of diagnostic tools and potentially enable future antigen-specific therapeutic strategies.