25 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 immunological 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 uptake was assessed by skin sensiti-
tion, 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 of Foxp3-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 re-
sponses 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.

26 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 UBV-induced immunosuppression may be useful in the management of skin cancers. Topical treatment of honokiol, a phytochemical from Magnolia plant, inhibits UBV-induced suppression of contact hyper-sensitivity (CHS) response in mice. Here, we report that inhibition of UBV-induced sup-
pression of CHS in C57BL/6 mice by treatment of honokiol (0.5 and 1.0 mg/cm² skin area) increased IFN-γ production in mouse peritoneal macrophages. Furthermore, administration of honokiol on mice prior to UBV irradiation significantly increased the CD8+ T cell activation rate compared to the control group. These results indicate that honokiol may have a role in stimulation of immune system in UVB-exposed animals.

27 Induction of pсорiasiform dermatitis in mice following cutaneous purinergic 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 approxi-
mately 2% of the US population. This disease is dependent on IL-17-secreting innate inflammatory cells and TH17 cells that are based by cutaneous DCs and likely keratinocytes. Several triggers have been proposed as stimulus 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 purinergic P2X7 receptor (P2X7R) signaling, induces NF-κB activation and the IL-23/IL-17 axis, both of which have been shown to be pсорiasis susceptibility pathways. We hypothesize that alarmins, such as ATP, play a pivotal role in the induction and maintenance of innate and adaptive cutaneous immune responses. Our hypothesis is supported by signaling through the P2X7R in the presence of ATP inhibition, induces pсорiasiform dermatitis in mice characterized by uniform immune responses in psoriasis. Here we report that signaling through the P2X7R, in the

28 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 (IFIs) suggests local recognition of both HLA-I and II 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-I and II epitopes. We derived a list of 313 HLA-I expressed proteins from our gene expression and proteomic data and

29 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), though the exact interactions of stress with AA remain undefined. Corticotropin-releasing hormone (CRH), the proinflammatory 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 PBMC 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=0.001). High CRHR1 expression was significantly related to chronic AA (disease duration >1year; p=0.001, χ² test), and large lesion area (AA >25%; p=0.027, χ² test). High CRHR1 was correlated to a low expression of PD-L1 (p=0.011) 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 upregu-
lation, 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 increasing cytokine expression during the 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 inmmunoregulatory cytokines remains unclear. To address this issue, we applied PD-L1 deficient mice to mimic contact hypersensitivity (CHS). PD-L1-deficient mice exhibited significantly exacerbated CHS responses with enhanced IFN-γ production from T cells in the skin. Administration of PD-1/PD-L1 pathway to modulate 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+ dermal 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 in a time-dependent manner. Furthermore, administration of PD-L1 pathway negatively regulates the development of CHS. PD-L1 on Langerin- dDCs may play a role to control CD8+ T cell activation in the skin.