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

H Mizutani1, R Yamagawa-Minesaka1, Y Minami1, K Yagita1 and N Katoh1 1 Department of Dermatology, Kyoto Prefectural University of Medicine, Kyoto, Japan, 2 Departments of Dermatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan, 3 Physiology and Systems Biochemistry, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

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 both for tolerance induction. Six weeks later, hapten was reapplied 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 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, Per2, also exacerbated the ear-swelling responses and cell infiltration accompanied by the decrease of FoxP3- regulatory T cells into inflamed skin in taken together, circadian rhythms may be involved in the immune tolerance induction in allergen inflammation.

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

SK Kyatai1, HC Pal2 and R Prasad3 1 University of Alabama at Birmingham, Birmingham VA Medical Center, Birmingham, AL and 2 University of Alabama at Birmingham, Birmingham, AL

Solar ultraviolet (UV) radiation induced immunosuppression has been implicated in skin cancer risk; therefore the treatment options which can inhibit UV-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 hypersensitivity (CHS) response in mice. Here, we report that inhibition of UVB-induced suppression of CHS in C57BL/6 mice by treatment of honokiol (0.5 and 1.0 mg/cm² skin area) was associated with reduced levels of DNA methylation as well as DNA methyltransferase activity in the mouse skin compared with non-honokiol-treated UVB-exposed control mice. Cell population responsible for the honokiol mediated inhibition of UVB-induced immuno-suppression was characterized by using an adoptive transfer approach: DNA-methylated dendritic cells (DCs), isolated from lymph nodes of donor mice that had been UVB-exposed and sensitized to 2,4-dinitrofluorobenzene (DNFB) with and without honokiol treatment, were transferred into naïve recipient mice. The CHS response of the recipient mice to DNFB was then measured. Honokiol treatment of UVB-exposed donor mice inhibited the suppression of the CHS response in naïve 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. The correlation of CHS response in mice receiving DCs from honokiol treated, UVB-exposed donor mice was also associated with enhanced secretion of Th1-type cytokines and T-cell stimulation compared with the Th1-type cytokines from the DCs obtained from non-honokiol-treated and UVB-exposed donor mice. These data suggest that correlation of DNA hypomethylation induction by honokiol may have a role in stimulation of immune system in UVB-exposed animals.

027 Induction of pсорiasiform dermatitis in mice following cutaneous purinergic P2X7 receptor signaling is dependent on neutrophil and inflammasome activation

JA Diaz-Chevez, ME Killeen and AR Mathers University of Pittsburgh, Pittsburgh, PA

Psoriasis vulgaris is a cell mediated inflammatory cutaneous disease that affects approximately 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 stimuli that induce DCs and keratinocytes to secrete IL-17-polarizing cytokines to generate the psoriasis phenotype. One potential stimuli is alarmins, such as ATP. ATP is a particularly interesting alarmin that, via purinergic P2X7 receptor signaling, induces NF-kB 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. Here, we report that signaling through the P2X7 receptor in the presence of ATP/LPS exposure, induces psoriasiform 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-1β, IL-23, S100A9, and IL-6 were also observed. Furthermore, the inflammatory response induced following P2X7 signaling in vitro is largely dependent on neutrophils and the IL-1/NLRP3 inflammasome pathway. In conclusion, our results demonstrate that cutaneous inflammatory responses induced via purinergic signaling through the P2X7R have implications in the pathogenesis and potential treatment of inflammatory diseases, such as psoriasis.

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

T Gordon1, E Wang1, A De Jong1, P Sinu1, CA Lindestam1, A Sette2 and AM Christiano3 1 T Columbia University, New York, NY, 2 La Jolla Institute for Allergy and Immunology, San Diego, CA and 3 Department of Dermatology and Genetics and Development, Institute of Human Nutrition, New York, NY

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-A1 and HLA-I bound autoantigens. Since growing (anagen) HFs are preferentially affected 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 -B8 epitopes. We derived a list of 313 HLA-A1 restricted peptides from our gene expression and proteomic data and publicly-available sources, with a specific emphasis on anagen stage proteins. We next employed the recently described HLA-I pH presentation and standard HLA-I allele specific prediction for common HLA-A variable 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 = 5.0 x 10e-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 tolerogenic therapies.

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

H Guo1, L Xu2, E Wang3 and KJ McEwan2 1 University of British Columbia, North Vancouver, Canada, 2 Children and Family Research Institute The University of British Columbia, Vancouver, Canada and 3 University of British Columbia, Vancouver, Canada

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 in monocytes was enhanced in AA compared with controls (3.17% versus 1.44%, p < 0.0001). High CRHR1 expression was significantly related to chronic AA (disease duration > 5years, p = 0.001, χ2 test), and large lesion area (AA > 25%; p = 0.027, χ2 test). High CRHR1 expression was related to a low T score (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 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 disease development during the course of disease. CRHR1 expression is associated with cutaneous inflammation in AA patients, but in CRH insensitive subjects, CRH may influence autoimmune inflammation by promoting monocyte migration and LTB production.

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

T Hirano1, T Honda2, K Tamaeda3, L Chen4 and K Kabashima1 1 Department of Dermatology, Kyoto Univ., Kyoto, Japan, 2 Department of Immunology, Yamaguchi Univ., Yamaguchi, Japan and 3 Department of Immunobiology, Yale Univ., New Haven, CT

PD-L1 is expressed on the bystander cells in the lesional inflammatory site, and its ligand 1 (PD-1) 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 PD-L1 blockade effectively abrogated PD-L1 blockade 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. We investigated the potential role 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 expression of PD-L1 during challenge. After the elicitation, the expression of PD-L1 on Langerin’ dDCs was reduced during the recovery phase. The regulatory mechanisms of the PD-1/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.

Adaptive and Auto-Immunity ABSTRACTS

www.jidonline.org $5