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Severe cutaneous HSV-2 infection at night time compared with at day time is due to upregulation of an HSV-2 receptor nectin-1 driven by CLOCK protein in mice

Y Ogawa[1], T Matsuzawa[1], S Shimada[1], A Nakao[2] and T Kawamura[1] *1 Dermatology, University of Yamanashi, Yamanashi, Japan and 2 Immunology, University of Yamanashi, Yamanashi, Japan*

Recent studies have shown that host responses to bacterial infection or endotoxin were dependent on time of day. However, it is unknown whether the time of herpes simplex virus 2 (HSV-2) infection affects the outcome. To demonstrate it, wild-type (WT) mice housed under 12-hour light/dark conditions (the light was turned on at Zeitgeber time [ZT] 0, and the light was turned off at ZT12) were intradermally infected with HSV-2 either at ZT6 (rest phase) or at ZT18 (active phase). Clinical severity and mortality were higher in mice infected at ZT18 than at ZT6. The viral titers in the skin were higher in mice infected at ZT18 than at ZT6. Next, to gain a mechanistic insight, we examined the kinetics of HSV-2 receptor nectin-1 (Pvrl1) as well as clock genes in the skin of WT mice without infection. mRNA levels of *Pvrl1* as well as clock genes *Per1-3*, *Cry1*, and *Bmal1* exhibited circadian variations. Moreover, at the protein levels, nectin-1 expression in the mouse skin was higher at ZT18 than at ZT6. Notably, the time-of-day-dependent variation in the severity of cutaneous HSV-2 infection was also observed in RAG2-deficient mice, suggesting that acquired immunity did not play a role. Pam 212 keratinocytes and normal human epidermal keratinocytes exhibited circadian oscillations in mRNA levels of *Pvrl1* as well as clock genes after synchronization by serum shock. Moreover, ChIP assays revealed that the circadian clock component CLOCK temporally bound to the promoter region of Pvrl1 in these cells. Our findings suggest that the time of HSV-2 infection affects the outcome, in association with circadian expression of HSV-2 receptor nectin-1 in the skin that is driven by the circadian clock system.

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An investigation of the molecular pathogenesis of Generalized Pustular Psoriasis and its overlap with Psoriasis Vulgaris

M Catapano[1,2], F Ciccarelli[1,2], J Barker[1] and F Capon[1] *1 Division of Genetics and Molecular Medicine, King's College London, London, UK and 2 The Francis Crick Institute, London NW1 1AT, UK*

Psoriasis is a complex, immune-mediated skin disorder that can be classified into several forms, including Psoriasis Vulgaris (Ps) and Generalised Pustular Psoriasis (GPP). While the genetic basis of Ps has been widely studied, the pathogenesis of GPP remains poorly understood. Genetic studies have identified GPP mutations in *AP1S3*, *CARD14* and *IL36RN*, indicating an involvement of innate, autoinflammatory pathways that appear to be distinct from those causing Ps. In this context, the aim of our study was to investigate the molecular pathogenesis of GPP and its overlap with Ps and autoinflammatory diseases (AID), using transcriptomic approaches. To achieve this purpose, we generated whole-blood RNAseq data on 9 GPP cases and 7 unaffected controls. We also accessed publicly available Ps and AID transcriptome datasets. We analysed all gene expression profiles using DESeq2, limma and Ingenuity Pathway Analysis (IPA). The analysis of the GPP whole-blood transcriptome identified 86 genes that were up-regulated in patients compared to controls (fold-change>1.5; FDR<0.05). Pathway enrichment analysis highlighted a strong type-I-IFN signature ($FDR=8.7x10^{-6}$), but only a marginal enrichment of IL-1 signalling genes ($FDR=4x10^{-2}$. Furthermore, the comparison of the GPP transcriptome with publicly available datasets demonstrated a significant overlap with conditions associated with excessive type-I-IFN production, but not with the genes that are up-regulated in Ps or in IL-1 driven autoinflammatory syndromes. In conclusion, although Ps and GPP are often described as part of the same psoriasis-spectrum, the analysis of their transcriptomes highlighted important differences. Further investigation of these results in larger datasets is expected to clarify the role of type I IFN in GPP and improve our understanding of disease mechanisms.

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Deficiency of *Bach2* results in improved tumor immunity by enhancing effector function in CD8 T cells

Y Sato[2], M Matsui-Watanabe[1], M Matsumoto[1], DR Sharda[3], K Igarashi[1] and R Okuyama[2] *1 Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan, 2 Dermatology, Shinshu University, Matsumoto, Japan and 3 Biological Sciences, Olivet Nazarene University, Bourbonnais, IL*

Bach2 is a transcription repressor which belongs to the basic region-leucine zipper family and binds to Maf-recognition elements (MAREs). *Bach2* plays essential roles in B cell development, immunoglobulin class-switch recombination and somatic hypermutation of immunoglobulin encoding genes. *Bach2* is also required for development of effector T cells and regulatory T cells. These findings suggest that *Bach2* plays important role in development, differentiation or function of various immune cells. We speculated that the deficiency of *Bach2* would result in an altered immune response in tumor rejection. A subcutaneous transplantation model revealed that the tumor transplanted into the *Bach2* KO mice grew more slowly than the wild-type (WT) mice. These observations suggested that tumor immunity in the *Bach2* KO mice was upregulated. We examined tumor-infiltrating cells with flowcytometry. The flowcytometric analysis revealed that significantly more $CD8^+$ T cells infiltrated into the tumors in *Bach2* KO mice than WT mice. The expression level of *Gzmb* and *Ifng* was elevated in tumor-infiltrating *Bach2* KO $CD8^+$ T cells than WT cells. An electrophoretic mobility-shift assay revealed that Bach2 bound to the MARE-like sequence of *FasL* and *Gzmb*. The binding of Bach2 to MARE-like sequence of *Gzmb* required the hetero-dimer formation with MafK. The analysis of DNA micro array and ChIP-seq revealed that Bach2 may represses the klra family which is important for activation of NK cells. These results suggest that Bach2 represses the effector function of $CD8^+$ T cells by repressing expression of effector genes directly and Klra family might be important for not only NK cells but also for $CD8^+$ T cells.

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Single cell analysis reveals diversity of phenotype and function of autoantigen-specific B cells in systemic sclerosis

A Yoshizaki[1], T Fukasawa[1], S Ebata[1], Y Asano[1], K Mawatari[2], T Kitamori[2] and S Sato[1] *1 Dermatology, The University of Tokyo Graduate School of Medicine, Tokyo, Japan and 2 Applied Chemistry, The University of Tokyo Graduate School of Engineering, Tokyo, Japan*

Systemic sclerosis (SSc) is a connective tissue disease with an autoimmune background. Several studies indicated that B cells play crucial roles in SSc, though the mechanism and pathogenesis of SSc remain unknown. The autoreactive B cells have the ability to produce several cytokines independent of an antibody-producing function, which results in the regulation of disease severity of SSc. However, the role of autoreactive B cells remains unclear, because the number of autoreactive B cells is too small to study their functions directly. In this study, we developed the original assay system, microfluidic ELISA system, which can detect extremely small amounts of analytes and realize the direct studies for single B cells. After topoisomerase (topo) I-specific B cells were purified from SSc patients, these B cells were separated into single cells. Sequentially, their cytokine productions were analyzed. We also studied the function of topo I-specific B cells in topo I and complete Freund's adjuvant-induced SSc model mice using adoptive transfer experiments. We found that topo I-specific B cells produced higher amounts of IL-6 compared with topo I-non-specific B cells. By contrast, topo I-specific B cells produced lower amounts of IL-10 compared with topo I-non-specific B cells. In the SSc model mice, there were high or low affinity topo I-specific B cells. High affinity topo I-specific B cells produced higher amounts of IL-6 compared with lower affinity topo I-specific B cells. These B cells increased skin and lung fibrosis when they were adoptively transferred to the SSc model mice. These findings show that autoantigen-specific B cells have important roles in SSc. In addition, our original microfluidics methods can reveal new etiology of SSc, which may lead the novel therapeutic strategies.

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Non-conventional T-cells in the pathogenesis of alopecia areata: Targeting iNKT10 cells with α-galactosylceramide

A Gilhar *Technion, Haifa, Israel*

Alopecia areata (AA) is now widely accepted as a CD8+/NKG2D+ T cell-dependent, IFN-γ-driven model autoimmune disease that selectively attacks growing (anagen) hair follicles (HFs). However, several other cell populations also express NKG2D+ and produce large amounts of IFN-γ. These include the recently discovered type 1 innate lymphocytes (ILC1) and a sub-group of natural killer T cells (NKT) named NKT10. Here, we have asked whether these non-conventional T cells, are involved in the pathogenesis of AA. Triple immunofluorescence microscopy showed the presence of several different subsets of non-conventional lymphocytes (iNKT, γδ T cells, ILC1, ILC2) around and/or in lesional HFs in AA patients. While essentially no ILCs were found in healthy normal skin, their number was significantly increased in lesional AA skin, namely that of ILC1 cells. A significant increased number of NKT10 cells were observed in lesional as compared to healthy skin. AA lesions can be rapidly induced in healthy human scalp skin xenotransplants on Beige/SCID mice by intradermal injection of autologous healthy-donor PBMCs pre-cultured with IL-2. Here we show that the development of AA lesions in this in vivo model can be prevented by administration of the autologous PBMCs pre-cultured in IL-2, but also with the recognized NKT cell activator, α-galactosylceramide (α-GalCer), which induced IL-10 production by iNKT cells. We also show that this protective mechanism required both iNKT cells and IL-10. Furthermore, hair regrowth even occurred in established xenograft AA lesions following treatment with either α-GalCer or exogenous IL-10. This identifies iNKT10 cells as novel key players in human AA and suggests that pharmacologically targeting iNKT10 cells is a promising novel management strategy for both, preventing and treating AA and possibly in other related human autoimmune diseases.

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Disclosing the interdependence: How obesity orchestrates skin disease

Y Skabytska and T Biedermann *Dermatology, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany*

The inflammatory skin disease psoriasis is highly prevalent and relevant. This disease is often characterized by comorbidities with obesity as the most prevalent. Clinical observations clearly indicate obesity as an aggravating factor for skin inflammation in psoriasis. The aim of the study was to investigate how obesity alters skin immune responses. To this end, a obesity model was used by feeding of C57Bl/6J mice with high-fat diet (HFD, 60% fat). To investigate the effect of obesity on Th17/Th1-mediated skin inflammation, a mouse model of 2,4,6-trinitrochlorobenzene (TNCB) contact hypersensitivity (CHS) was used. Challenging sensitized mice resulted in an increased ear swelling of obese mice. Interestingly, the ear swelling of Th2-mediated CHS against fluorescein isothiocyanate (FITC) was strongly decreased. Characterization of the immune response in the skin of TNCB-challenged mice revealed a significant upregulation of IL-17 and unchanged other cytokines, among them IL-4 and IL-13, in obese mice. In draining lymph nodes, we found an increase of CD4+ and CD8+ T cells with a decrease of relative numbers of other cells, indicating that T cells were effector cells responsible for aggravation of skin inflammation by obesity. The characterization of cell phenotypes showed an increase of IL-17+TNF+ cells and IFNγ-producing CD8+ T cells. Interestingly, the elevation of T cell numbers and TNF/IL-17 production in blood occurred already before TNCB sensitization, indicating systemic inflammation promoted by obesity. Next we tested whether systemic factors, increased due to HFD, are responsible for alterations in cell phenotypes. Treatment of immune cells with serum from obese mice resulted in an enhanced production of TNF by Gr1+ cells and elevated IL-17-producing CD4+ and CD8+ cells. The incubation of T cells with serum from obese mice during different polarization conditions led to a promotion of Th1 phenotype with a marked reduction of Tregs. Taken together, our work indicates that obesity promotes skin inflammation by selective enhance of Th17/Th1 immune response.