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Upregulation of miR-941 in peripheral CD14+ monocytes enhances osteoclast activation and osteolysis in patients with psoriatic arthritis: a potential diagnostic biomarker and treatment target

S Lin[1,2], C Lee[1,2] and C Hong[3,4] *1 Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, 2 College of Medicine, Chang Gung University, Taoyuan, Taiwan, 3 Dermatology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan and 4 Dermatology, National Yang Ming University, Taipei, Taiwan*

In psoriatic arthritis (PsA), progressive bone destruction was mediated by osteoclasts differentiated from monocyte. MicroRNAs (miRNAs) regulate many pathophysiological processes, however, how they regulate osteoclast activation and bone resorption has not been examined. This study aims to address whether specific miRNAs in CD14+ monocytes and osteoclasts derived from them cause active osteoclastogenesis in PsA. The RNA from circulatory CD14+ monocytes was isolated from PsA patients, psoriatic patients without arthritis, and normal controls (NCs). The miRNA expressions were profiled by next-generation sequencing. The candidate miRNAs were validated by PCR in 32 PsA patients and 31 NCs. Osteoclasts were induced from CD14+ monocytes by TNF-α and RANKL. Osteoclast differentiation and bone resorption were measured by TRAP immunostaining and dentin slice resorption, respectively. The results showed that miR-941 was selectively upregulated in CD14+ monocytes from PsA patients. Activation and bone resorption were enhanced in osteoclasts from PsA patients, but both were abrogated by RNA interference against miR-941. After successful biologic treatment, the increased miR-941 expression in CD14+ monocytes from PsA patients was abrogated. However, osteoclastogenesis and resorption remained increased. Collectively, our findings suggest that miR-941 could be an early diagnostic potential biomarker, a dormant mediator leading to recurrence, and treatment niche for PsA.

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Assessment of eosinophils degranulation in skin in drug reaction with eosinophilia and systemic symptoms (DRESS)

F Dezoteux[1,2,4], J Gibier[3], B Lopez[5,2], A Bongiovanni[6], G Lefèvre[5,2] and D Staumont-Sallé[1,2,5] *1 Dermatology Department, CHU Lille, Lille, France, 2 CEREO, National Reference Center for Hypereosinophilic Syndrome, Lille, France, 3 Institute of Pathology, CHU Lille, Lille, France, 4 University of Lille, Lille, France, 5 Laboratory of Immunology and Clinical Immunology Unit, CHU Lille, Lille, France and 6 Bioimaging Center, University of Lille, Lille, France*

BACKGROUND: DRESS for "Drug Reaction with Eosinophilia and Systemic Symptoms" is a drug-induced hypersensivity syndrom that can lead to severe organs failure. The role of eosinophils (Eo) in tissue damage remains unknown. We aimed to analyze the activation status of cutaneous Eo in DRESS. METHOD: Paraffin-embedded skin biopsies of patients from a French University hospital were retrospectively collected. We included 40 patients (2 groups of drug reaction): 19 MPE (maculopapular exanthema) and 21 DRESS. Eo and extracellular eosinophil granules were identified by immunohistochemical staining of eosinophilic granule proteins (ECP, MBP). The cell quantification was performed following 2 methods: 1) a manual method focused on 3 HPFX20 and scored according to the literature; 2) an original semi-automatic method after digital acquisition of a whole section. RESULTS: The number of Eo was not significantly different between EMP and DRESS patients, whatever the method of quantification. Whereas extracellular eosinophil granules were more frequent in the DRESS group ($n = 13/21$, 61.9%, $p = 0.009$) and predominant with the anti-MBP staining compared to anti-ECP staining ($p = 0.036$) that highlighted the presence of cutaneous activated Eo in DRESS compared with MPE. Moreover, the semi-automatic quantification method was more exhaustive and sensitive with twice more detected cells/mm^2 compared to the manual method. CONCLUSION: Our study suggests that cutaneous Eo are more activated in patients with DRESS compared with patients with MPE, according to the number of extracellular granules. We also developed an original and objective method of eosinophils and extragranular granules. Further study will be necessary to explore Eo recruitment mechanisms in tissues.

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Pathophysiology of desmoglein antibody-negative pemphigus vulgaris

K Amber[2], A Chernyavsky[1], A Agnoletti[1], C Wang[1] and SA Grando[1] *1 Dermatology, UC Irvine, Irvine, California, United States and 2 University of Illinois, Chicago, Illinois, United States*

Pemphigus vulgaris (PV) is a potentially lethal mucocutaneous blistering disease characterized by IgG autoantibodies (AuAbs) binding to epidermal keratinocytes and inducing a devastating mucocutaneous blistering disease. In this study, we showed that he non-desmoglein (Dsg) AuAbs present in the serum of the Dsg1/3 AuAb-negative acute PV patients are pathogenic, since patients' IgGs induced skin blistering in neonatal mice due to suprabasal acantholysis. The serum level of AuAbs to desmocollin (Dsc)3, M3 muscarinic acetylcholine receptor (M3AR) and the secretory pathway Ca^{2+}/Mn^{2+}-ATPase isoform 1, (SPCA1) encoded by the *ATP2C1* gene correlated with the disease stage of PV, and absorption of recombinant Dsc3, M3AR or SPCA1 prevented both skin blistering in the passive transfer of AuAbs model of PV in BALB/c mice and significantly decreased extent of acantholysis in the neonatal mouse skin explant model. While the acantholytic activities of each of these immunoaffinity purified AuAbs were insufficient to induce PV-like phenotype in vivo, their mixture produced a synergistic effect manifested by positive Nikolskiy sign in the skin of neonatal mice. A computer simulation of the PV-associated HLA polymorphism DRB1*04:02 identified potential immunogenic epitopes of these non-Dsg antigens with avidities for specific binding of HLA−peptide complexes to T-cell receptors that were similar to those of the immunogenic portion of Dsg 3. The downstream signaling of all pathogenic non-Dsg AuAbs involved p38 MAPK phosphorylation and elevation of cytochrome c and caspase 9. Anti-Dsc3 and anti-SPCA1 AuAbs also activated Src. Thus, while multiple hits sustained by a constellation of non-Dsg AuAbs appeared to be sufficient to disrupt epidermal integrity, elimination of a single type of pathogenic AuAb was sufficient to prevent keratinocyte detachment and blistering. Therefore, anti-Dsg1/3 AuAb-free PV can be a model for elucidation role of AuAbs to non-Dsg antigens in the physiological regulation of keratinocyte cell-cell adhesion and blister development.

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IL-27 in macrophages mediates T cell survival and dermal cluster formation in allergic contact hypersensitivity

J Suwanpradid[1], P Hoang[1], J Kwock[1], L Floyd[1], J Smith[1], R Kedl[2], A Atwater[1], S Rajagopal[1], D Corcoran[1] and AS MacLeod[1] *1 Duke University, Durham, North Carolina, United States and 2 University of Colorado Denver, Aurora, Colorado, United States*

Re-exposure to a relevant skin allergen initiates the efferent phase and clinical expression of allergic contact dermatitis (ACD) characterized by local DC-mediated T cell activation and recruitment of Ag-specific T cells to the sites of allergen challenge. We here made the novel observation that, in contrast to non-lesional skin of ACD patients, skin of (+) patch-tested individuals (effector phase of ACD) showed high expression of IL-15 in epidermal keratinocytes and in dermal DC-macrophage-T cell- clusters. Interestingly, within these clusters, T cells with increased anti-apoptotic BCL2 were found directly adjacent to IL-27-producing CD14$^+$CD86$^+$CD172a$^+$ cells and IL-15$^+$ cells. Moreover, in the allergic contact hypersensitivity (CHS) model using IL-27p28^{eGFP} mice, we found increased IL-27 production in a distinct macrophage subset expressing CD172a. Functionally, IL-27 conditional knockout mice (driven by LysM-cre and CD11c-cre promoters) demonstrated suppressed DNFB-induced ear thickening ($p < 0.05$) supporting a role for IL-27 in CHS. Mechanistically, IL-27 stimulated *IL15* in human epidermal keratinocytes in a STAT-1 dependent manner as evidenced by rapid p-STAT1 nuclear translocation and abrogation of this response by silencing STAT1 ($p < 0.05$). Given the relevance of the recently identified allergen-induced dermal T cell clusters for CHS responses, we tested the functional relevance of IL-27-induced IL-15 signaling in this context. In the CHS mouse model, administration of IL-27-neutralizing antibody (i.d.) resulted in decreased *IL-15* expression associated with downregulation of BCL2 in T cells, the number of total cutaneous CD8$^+$ T cells and T cell clusters ($p < 0.05$). Similarly, when treated with recombinant IL-15, human skin T cells increased BCL2 expression ($p < 0.05$). Overall our findings implicate IL-27 as a potential therapeutic agent in regulating cutaneous T cell immunity.

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The effect of matrix metalloproteinases-3 on the deposition of α2AP in systemic sclerosis

H Niwa[1], Y Kanno[1,2], E Shu[1], H Kanoh[1] and M Seishima[1] *1 Dermatology, Gifu University, Gifu, Japan and 2 Clinical Pathological Biochemistry, Faculty of Pharmaceutical Science, Doshisya Women's College of Liberal Arts, Kyoto, Japan*

Alpha2-antiplasmin (α2AP) is the major circulating inhibitor of plasmin, which plays an important role in the regulation of intravascular fibrinolysis. We previously showed that the expression of α2AP was elevated in dermal fibroblasts obtained from patients with systemic sclerosis (SSc), and α2AP is associated with the development of fibrosis in SSc. In SSc, the deposition of α2AP may play an important role in the development of fibrosis. Matrix metalloproteinases-3 (MMP-3), which is one of the extracellular matrix (ECM)-degrading enzymes is known to cause the dysfunction of α2AP by cleaving the Pro19-Leu20 peptide bond in α2AP. In the present study, we focused on MMP-3, and examined the relationship between α2AP and MMP3 in SSc. Although there is no difference in the serum levels of α2AP and MMP3 between healthy controls and SSc patients, the expression of α2AP was elevated, and the ratio of MMP-3 and tissue inhibitors of metalloproteinase-1 (TIMP-1) was decreased in SSc dermal fibroblasts. Next, we examined the effect of MMP-3 on the deposition of α2AP in SSc dermal fibroblast, and showed that MMP-3 promoted the degradation of α2AP and reversed a profibrotic phenotype of SSc dermal fibroblasts. Furthermore, we showed that microRNA-29a (MiR-29a), which reduces TIMP-1 production attenuated the SSc-induced α2AP expression. Our findings suggest that MMP-3 plays a pivotal role on the α2AP-associated dermal fibrosis, and might contribute to a novel therapeutic approach for SSc.

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Dynamics of alpha beta T cell accumulation in human fetal skin

MO Dhariwala[1], KS Vasquez[2,1], ML Pauli[1], M Rosenblum[1] and TC Scharschmidt[1] *1 Dermatology, UCSF, San Francisco, California, United States and 2 Stanford University, Palo Alto, California, United States*

Alpha beta (αβ) T cells in adult skin play a critical role in defense against infections and malignancy while also promoting homeostatic functions such as wound repair. Comparatively little is known about the phenotype and function of these cells during human skin development. Here we performed flow cytometry on fetal skin ranging from 17 to 23 weeks gestational age (GA) in order to interrogate the early dynamics of αβ T cell accumulation in human skin and elucidate phenotypic differences between these fetal populations and their adult counterparts. We found that αβ T cell numbers increase significantly during the second trimester of fetal life. While the percentage of live CD3$^+$ cells remained substantially lower in fetal as compared to adult skin, the CD4:CD8 ratios were comparable across all ages. Notably, as has been seen in pediatric skin, the percentage of Foxp3$^+$CD25$^+$ cells was elevated in skin at 23 weeks GA vs. adulthood, suggesting an early enrichment of regulatory T (Treg) cells. All fetal αβ T cells demonstrated high Ki-67 and CD31 expression, consistent with active seeding of the tissue by recent thymic emigrants with a high proliferative capacity. Fetal Foxp3^{neg}CD4$^+$ and CD8$^+$ T cells demonstrated a naïve cell phenotype with low surface expression of the memory marker CD45RO and less production of IL17A, IL-13, IL-22 and TNFα upon restimulation as compared to their adult skin counterparts. In contrast, fetal Tregs at 23 weeks GA already expressed high levels of CD45RO as well as key activation markers such as ICOS, Foxp3 and CD25. Tregs are known to preferentially localize to hair follicles in adult skin. Notably, Tregs increased in skin between 19 and 23 weeks GA, coincident with terminal hair-follicle development, and were enriched in scalp as compared to torso skin within a given fetus. Taken together our data support early establishment of a memory-like Treg population in human fetal skin, which may support tolerance during recognition of self and potentially exogenous antigens in fetal and postnatal life.