Uregulation of mir-941 in peripheral CD14+ monocytes enhances osteoclast activation and osteolysis in patients with psoriatic arthritis: a potential diagnostic biomarker and therapeutic target

S Lin1, C Lee2,1 and C Hong2,1 1 Dermatology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan, 2 College of Medicine, Chang Gung University, Taoyuan, Taiwan, 3 Dermatology, Kaohsiung Veters General Hospital, Kaohsiung, Taiwan, 4 Dermatology, National Yang Ming University, Taipei, Taiwan

In psoriatic arthritis (PsA), progressive bone destruction was mediated by osteoclasts differentiated from monocyte. MicroRNA(s) (miRNAs) regulate many pathological 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 qPCR 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 tartrate-resistant acid phosphatase (TRAP) staining, reverse zymography, 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 an early dysregulation of a potential biomarker, a dormant mediator leading to recurrence, and treatment niche for PsA.

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

J Guise1,2, J Hsiung1,2, J Borregoavn1, G Lefèvre3,2 and D Staumont-Salle1 1 Dermatology Department, CHU Lille, Lille, France, 2 CERE, National Reference Center for Hypersensitivity Syndrome, Lille, France, 3 Institute of Pathology, CHU Lille, Lille, France

ABSTRACTS | Adaptive and Auto-Immunity

Pathophysiology of desmoglein antibody-negative pemphigus vulgaris
K Amber1, A Chernyavsky1, A Agnoletti1, C Wang1 and SA Grando1 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 keratocytomes and inducing a devastating mucocutaneous blistering disease. In this study, we showed that non-dsg1 and dsg3 AuAbs present in the serum of the Digi/3/AuAbs-negative acute PV patients are pathogenic, since patients’ IgGs induced skin blistering in neonatal mice due to superab acantholysis. The serum level of AuAbs to desmocollin (Dsc3), M3 muscarinic acetylcholine receptor (M3AR) and the secretory pathway Ca2+/Mn2+-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 and significantly extent of acantholysis in a neonatal mouse skin explant model. While the acantholytic activity of each of these immune complexes purified AuAbs were insufficient to induce PV-like phenotype in vivo, their mixture produced a synergistic effect manifested by positive Nikolsky 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-α peptide complexes to T-cell receptors that were similar to those of the immunogenic portion of Dsg 1. 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 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-Dgi/3/AuAbs-free PV can be a model for elucidation of AuAbs to non-Dsg antigens in the physiological regulation of keratocytome-cell-cell adhesion and blister development.

The effect of matrix metalloproteases-3 on the deposition of z2AP in systemic sclerosis
H Niwa1, Y Kanno1,2, S Shi1, H Kanoh1 and M Seishima1 1 Dermatology, Gifu University, Gifu, Japan and 2 Clinical Pathological Biochemistry, Faculty of Pharmaceutical Science, Doshisya Women’s College of Liberal Arts, Kyoto, Japan

Alpha2macroglobulin (z2AP), the major circulating inhibitor of plasmin, which plays an important role in the regulation of intravascular fibrinolysis. We previously showed that the expression of z2AP was elevated in dermal fibroblasts obtained from patients with systemic sclerosis (SSc), and z2AP is associated with the development of fibrosis in SSc. In SSc, the deposition of z2AP may play an important role in the development of fibrosis. Matrix metalloproteases-3 (MMP-3), which is one of the extracellular matrix (ECM)-degrading enzymes is known to cause the dysfunction of z2AP by cleaving the Pro19-Leu20 peptide bond in z2AP. In the present study, we focused on MMP-3, and examined the relationship between z2AP and MMP-3 in SSc. Although there is no difference in the serum levels of z2AP and MMP-3 between healthy controls and SSc patients, the expression of z2AP was elevated, and the ratio of MMP-3 to z2AP of the inhibitory fibers of metalloproteinase-1 (TIMP-1) was decreased in SSc dermal fibroblasts. Next, we examined the effect of MMP-3 on the deposition of z2AP in SSc dermal fibroblast, and showed that MMP-3 promoted the degradation of z2AP and reverses a profibrotic phenotype of SSc dermal fibroblasts. Furthermore, we showed that microRNA-29a (miR-29a), which reduces TIMP-1 production, abrogated the SSc-induced z2AP expression. Our findings suggest that MMP-3 plays a pivotal role on the z2AP-associated dermal fibrosis, and might contribute to a novel therapeutic approach for SSc.

IL-27 in macrophages mediates T cell survival and dermal cluster formation in allergic contact hypersensitivity
J Surunpradit1, P Hong1, J Kwok1, J Floyd1, J Smith1, K Redd1, A Alvare1, S Rajagopal1, D O’Shea1, J Corcoran1 and AS MacLeod1 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 effenter 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-17 in epidermal keratinocytes and in dermal macrophage-T cell clusters. Interestingly, within these clusters, T cells with increased anti-apoptotic BCL2 were found directly adjacent to IL-27-producing DCs (IL-27'). Functionally, IL-27' conditional knockout mice (driven by Ly5m-cre and CD11c-cre promoters) demonstrated suppressed DNFB-induced ear thickening (p < 0.05) supporting a role for IL-27 in CHS. Mechanically, IL-27' stimulated IL-15 in human epidermal keratinocytes in a STAT1-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). Finally, 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.

Dynamics of alpha beta T cell accumulation in human fetal skin
MO Dhurjati1, KS Vasquez1,2, M Pauli1, M Roserblum1 and TC Scharschmidt1 1 Dermatology, UCSF, San Francisco, California, United States and 2 Stanford University, Palo Alto, California, United States

Alpha beta 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 skin fetal ranging from 17 to 21 weeks gestational age (GA) in order to interrogate the early dynamics of z2AP T cell accumulation in human skin and elucidate phenotypic differences between these fetal populations and their adult counterparts. We found that z2AP T cell numbers increase significantly during the second trimester of fetal life. While the percentage of live CD3+ T cells remained substantially lower in GA 17-21 weeks compared to adults, T cell numbers were comparable across all ages. Notably, as has been seen in pediatric skin, the frequency of FOXP3+CD25+ cells was elevated in skin at 23 weeks GA vs. adulthood, suggesting an early enrichment of regulatory T cells during fetal life. Furthermore, we demonstrated that IL-27 induced high IL-12 and IL-23 expression, consistent with active seeding of the tissue by recent thymic emigrants with a high proliferative capacity. Fetal FOXP3+CD25+ and CD8+ T cells demonstrated a naive cell phenotype with low surface costimulation and low production of TNFα and IFNγ. While FOXP3+CD8+ T cells were detected in fetal skin, we found increased IL-15 production in a distinct macrophage subset expressing CD172a. Functionally, IL-27' conditional knockout mice (driven by Ly5m-cre and CD11c-cre promoters) demonstrated suppressed DNFB-induced ear thickening (p < 0.05) supporting a role for IL-27 in CHS. Mechanically, IL-27' stimulated IL-15 in human epidermal keratinocytes in a STAT1-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). Finally, 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.

012 Dynamics of alpha beta T cell accumulation in human fetal skin

Dynamics of alpha beta T cell accumulation in human fetal skin

Dynamics of alpha beta T cell accumulation in human fetal skin