007 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

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In psoriatic arthritis (PsA), progressive bone destruction was mediated by osteoclasts differentiated from monocyte. MicroRNAs (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 osteoastrogensis 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 in vitro. 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 and potential biomarker, a dormant mediator leading to recurrence, and treatment niche for PsA.

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

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BACKGROUND: DRESS for “Drug Reaction with Eosinophilia and Systemic Symptoms” is a drug-induced hypersensitivity syndrome that can lead to severe organ failure. The role of eosinophils (Eo) in tissue damage remains unknown. We aimed to analyze the activation status of cutaneous Eo in DRESS. METHOD: Parallel-immunobed skin biopsies of patients from a French national registry 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 eosinophil granule proteins (ECP, MBP). The cell quantification was performed following 2 methods: a) a manual method focused on 3 HPFX20 and scored according to the literature; b) an original semi-automatic method after digital acquisition of a whole section. RESULTS: The number of Eo was not significantly different between MPE and DRESS patients, whatever the method of quantification. Whereas extracellular eosinophil granules were more frequent in the DRESS group (r = 0.321, 61.9%, p < 0.009) and predominant with the anti-MBP staining compared to anti-ECP staining (p = 0.016) 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 as many detected cells/mm² 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 demonstrated an original and objective method for eosinophils and extracellular granules. Further study will be necessary to explore Eo recruitment mechanisms in tissues.

009 Pathophysiology of desmoglein antibody-negative pemphigus vulgaris

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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 non-dermatitis PV AuAbs present in the serum of the Dg3/H1 AuAbs-negative acute PV patients are pathogenic, since patients’ IgG induced skin blistering in neonatal mice due to supra-basal 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 activation of recombinant Dsc3, M3AR or SPCA1 prevented both skin blistering in the passive transfer of AuAbs model in BALB/c mouse and a significantly increased extent of acantholysis in the neonatal mouse skin explant model. While the acantholytic activities of each of these immunoinflammatory AuAbs were insufficient to induce PV-like phenotype in vivo, their mixture produced a synergistic effect manifested by passive 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–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 p18 MAPK phosphorylation and elevation of cytochrome c and caspase 9. Anti-Dsg3 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, 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 and potential biomarker, a dormant mediator leading to recurrence, and treatment niche for PsA.

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

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Allergic contact dermatitis (ACD) is 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 (AHS) model using 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. 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). Importantly, 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.

011 The effect of matrix metalloproteases-3 on the deposition of zAP in systemic sclerosis

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Alpha2macroglobulin (a2M) 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 zAP was elevated in dermal fibroblasts obtained from patients with systemic sclerosis (SSc), and zAP is associated with the development of fibrosis in SSc. In SSc, the deposition of zAP 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 zAP by cleaving the Pro91-Leu94 peptide bond in zAP. In the present study, we focused on MMP-3, and examined the relationship between zAP and MMP-3 in SSc. Although there is no difference in the serum levels of zAP between healthy controls and SSc patients, the expression of zAP was elevated, and the ratio of cleaved and non-cleaved zAP in patients with high HR and CD11 expression, consistent with active seeding of the tissue by recent thymic emigrants with a high proliferative capacity. Fetal fibroblasts and CAF fibroblasts demonstrated a naive cell phenotype with low surface expression of a2M memory receptor, and less production of a2M and TNFα upon stimulation 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. 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 antigenic antigens in fetal and postnatal life.