Characterization of the B cell infiltrate in discoid lupus

MB Rapp1, N Bahkowskii1, F Essa3, T Cartan1, JH Andik1 and CT Richardson4,5
1 University of Buffalo, Buffalo, New York, United States, 2 University of Rochester, Rochester, New York, United States, 3 School of Medicine and Dentistry, University of Rochester, Rochester, New York, United States, 4 Dermatology, University of Rochester, Rochester, New York, United States and 5 Allergy, Immunology, and Rheumatology, University of Rochester, Rochester, New York, United States

Discoid lupus erythematosus (DLE) is a severely disfiguring and difficult to treat autoimmune skin disease for which no new therapies have been FDA-approved in over 60 years. One potential therapeutic target is cutaneous B cells. Though rare in healthy skin, B cells are prevalent in DLE lesions, comprising 10–20% of the robust lymphocytic infiltrate. However, the phenotype and function of this expanded B cell population have not been evaluated in detail and it is unknown whether cutaneous B cells play a role in DLE pathogenesis. We analyzed T cells (CD3), B cells (CD20), and plasma cells (CD138) by immunohistochemistry in skin biopsies from seventeen patients with a clinical and pathologic diagnosis of discoid lupus. Consistent with known literature, T cells predominated the infiltrate (180.3 cells/hpf, range 88-299). However, the number of B cells was not insignificant and comprised an average of 21% of the total lymphocytic infiltrate (39.3 cells/hpf, range 4-147). In contrast, very few plasma cells were noted in the biopsies (4.7 cells/hpf). The majority of the T and B cells analyzed were associated with one another in large perivascular and peridnexal clusters. Interestingly, the larger the total lymphocytic infiltrate, the greater the percentage of that infiltrate was comprised of B cells (p<0.0185). This correlation with increased inflammation suggests that cutaneous B cells may be playing more than a bystander role in DLE pathogenesis. In support of this hypothesis, analysis of this B cell infiltrate by immunofluorescence microscopy revealed a greater number of class-switched memory B cells (IgG+) than naive B cells (IgD+). This constitutes the first report of cutaneous memory B cells in DLE disease.

IQGAP3 is involved in keratinocyte response to inflammation

A Zolotarenko and S Bruskin
Laboratory of functional genomics, Vavilov Institute of General Genetics, Moscow, Moscow, Russian Federation

IQGAP proteins mediate many processes important for the development of hyperproliferative skin diseases, namely the EGF signaling, WNT and MAPK kinase cascades, they are required for cell adhesion and migration processes, tight junction and zonula occludens formation, cell cycle regulation. However, the possible contribution of the IQGAP3 in the development of hyperproliferative psoriatic plaques is unknown. We have shown earlier that IQGAP3 is overexpressed in the lesional skin of patients with psoriasis. Next we have performed the keratinocyte stimulation with proinflammatory cytokines associated with the development of hyperproliferative psoriatic plaques (TNF alpha or a mix of IL-17+TNF alpha+IFN gamma in physiological concentrations) or with non-specific skin irritant 12-O-tetradecanoylphorbol-13-acetate (TPA) or with the EGF growth factor. We have shown that 24h later both the proinflammatory cytokines and EGF stimulation led to the moderate elevation of the IQGAP3 expression (1.6 and 1.8 times respectively, p<0.05) unlike the TPA stimulation, which haven’t led to any significant alterations in the IQGAP3 expression. As we have earlier identified FRA1, one of the API transcription factors, to mediate keratinocyte activation in psoriasis, we were interested in the identification of a possible link to the IQGAP3 expression. Using a stable keratinocyte line overexpressing FRA1, we have shown that both the FRA1 overexpression itself or the overexpression under the proinflammatory conditions (IL-17+TNF alpha+IFN gamma stimulation) have led to the elevated expression of the IQGAP3 (3 and 4.8 times respectively, p<0.05). Thus the IQGAP3 overexpression in psoriatic plaques could be the consequence of the FRA1 overexpression under the proinflammatory conditions present in skin of patients with psoriasis. The research was supported by RSF (project 18-73-00126).

Disregulation of antioxidant enzyme PRDX5 in alopecia areata

AR Abdelaziz1, M Mani1, SO Ersuev1, S Gelfman1, P Lin1, I Ionita-Laza1, L Petukhova1 and A Christianso1
1 Genetics & Development, Columbia University, New York, New York, United States, 2 Dermatology, Columbia University, New York, New York, United States, 3 University of Okara, Punjab, Pakistan and 4 Biostatistics, Columbia University, New York, New York, United States

We previously published a genome-wide association study (GWAS) and meta-analysis to search for common alleles that contribute to risk of AA, and identified several genomic regions harboring potential susceptibility genes. One candidate susceptibility gene expressed in the hair follicle (HF) in AA is peroxiredoxin 5 (PRDX5) (p-value of 8.7 x 10^-15), which is also a GWAS gene in Crohn’s disease, sarcoidosis, and psoriasis. PRDX5 is a member of the family of antioxidant enzymes that are crucial for regulating oxidative stress. Our lab has completed sequencing of 897 whole exomes with a custom capture region of genomic sequencing. Using a chi-square or ‘goodness of fit’ test of variant enrichment we identified variants that are significant in both our GWAS and exome studies, thus likely candidate causal variants. Using Bayesian fine mapping, we found a GWAS and exome significant variant, rs574087, is expected to be a causal variant in keratinocytes and melanocytes (with a posterior inclusion probability index or PIP score greater than 0.1, lending to high likelihood of causality). To functionally validate our in silico studies, we immunostained healthy human HF and AA affected HF and found PRDX5 is upregulated AA human HF. PRDX5 is expressed in cultured melanocytes by immunostaining, which also was expected since melanocytes are known to have high levels of oxidative stress. We postulate that PRDX5 is crucial for protection from oxidative stress and its dysregulation can ultimately lead to autoimmunity. Our findings establish a connection between PRDX5 and causal variants, which provides a functional framework for further fine mapping define the role of PRDX5 in AA disease pathogenesis.