AI-14 REPOSITORY CORTICOTROPIN INJECTION (H.P. ACTHAR[®]) REVERSES CRITICAL ELEMENTS OF THE TLR9/ANTI-IGM RESPONSE IN HUMAN B CELLS IN VITRO

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Background Signaling through Toll-Like Receptor 9 (TLR9) is a central event in the activation of normal B cells and in the production of pathogenic autoantibodies in diseases such as systemic lupus erythematosus (SLE). We sought evidence for direct effects of repository corticotropin injection (RCI; H.P. Acthar[®] Gel), an FDA-approved treatment for selected cases of SLE, on isolated human B lymphocytes activated *in vitro* by engagement of TLR9 and the B cell receptor.

Methods CD19+ B cells from healthy volunteers (n=3) were activated *in vitro* with the TLR9 ligand ODN 2395 and anti-IgM. RCI was added to cultures at concentrations over a 20-fold range (controls received equal volumes of placebo gel). Messenger RNA was isolated from cells after one day in culture and cDNA libraries were prepared for multiplexed high-throughput sequencing on an Illumina HiSeq 2500. Analyses of RNA-Seq reads utilized Illumina CASAVA pipeline Version 1.8. RUVSeq R package v3.1 and edgeR were used to identify differentially expressed genes in paired comparisons between unstimulated and TLR9/anti-IgM activated cells and between activated cells treated with RCI and placebo.

Results Treatment of B cells in steroid-free medium with ODN 2395/anti-IgM- resulted in significant, reproducible induction of 162 distinct mRNAs (mean induction=8.87 ±0.95 fold; range=2.5–118.3-fold) and suppression of 80 mRNAs (mean suppression to 21.8%±0.8% of baseline; range=6% to 39% of baseline). RCI treatment resulted in significant, reproducible suppression of 13 of the ODN 2395/αIgM -induced mRNAs (mean suppression to 23.6%±3.1% of stimulated value; range 9.9% to 41.2%). The RCI-suppressed mRNAs included two key regulators of class switch recombination, AICDA and BATF3. RCI treatment resulted in significant, reproducible induction of 5 of the ODN 2395/αIgM -suppressed mRNAs (mean induction by RCI=7.65±2.34 fold; range=4.7 to 16.9-fold). The RCI-induced mRNAs included SLAM family member Ly9, a cell surface receptor capable of inhibiting autoantibody responses. No ODN 2395/αIgM -induced mRNAs were further increased after RCI treatment and no ODN 2395/αIgM -suppressed mRNAs were further reduced after RCI treatment (p=0.0002; Fisher's exact test).

Conclusions RCI treatment of human B cells cultured under steroid-free conditions and activated with TLR9 agonist ligand and B cell receptor stimulation resulted in reversal of key elements of the mRNA activation response. Whether these effects are related to clinical actions of RCI in SLE will be of interest for future investigation.

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AI-15 PATHOGENESIS OF LUPUS DERMATITIS: DIFFERENT SKIN-RESIDENT DENDRITIC CELL SUBSETS EXHIBIT DIFFERENT MIGRATION PATTERNS, UTILIZE DIFFERENT MECHANISMS OF MIGRATION, AND PLAY DIFFERENT ROLES

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Background Pathogenesis of lupus dermatitis is not clear. Skin contains two subsets of dendritic cells (DC) that express langerin: Langerhans cells (LC) reside in the epidermis; they induce tolerance to skin autoantigens in lupus-prone MRL-lpr and MRL+/+mice (King et al, 2015). Another subset resides in the dermis, langerin⁺ dermal DCs (LangdDC). Here, we examined: a) the migration pattern, b) mechanisms of migration, and c) the effect of modulating the migration and of depletion of these two skin-DC subsets in lupus dermatitis.

Methods First, we tracked the *in vivo* migration of skin-DCs at the steady state using Langerin-driven eGFP knock-in mice as well as by applying fluorophores (FITC/TRITC) to the skin of MRL and control mice. Second, we treated MRL mice with glycolipid αGalCer that ameliorates lupus dermatitis and determined its effect on the migration of LC and LangdDC. Third, we investigated cellular and molecular mechanisms of skin-DC migration using knockout mice and blocking antibodies. Finally, we used diphtheria toxin receptor knock-in MRL mice to conditionally ablate LC and/or LangdDC, and determined disease scores.

Results At the steady state, LCs were reduced, whereas Lang⁺dDCs were increased in the skin-draining lymph nodes of MRL-lpr and MRL+/+mice as compared to controls. *In vivo* tracking of cells using both eGFP knock-in mice and fluorophore application revealed a reduced migration of LCs but increased trafficking of LangdDCs to skin-draining lymph nodes in MRL mice. Such altered pattern of migration of these two skin-DCs was corrected by αGalCer treatment. However, αGalCer did not act on LCs through its well-known target iNKT cells but increased epidermal γδ T-cells that increased LC migration *in vitro*. The role of γδ T-cells in modulating LC migration was confirmed using knockout animals. CD40L deficiency or antibody blockade abrogated the ability of γδ T-cells to enhance LC migration. Finally, conditional ablation of LCs worsened lupus dermatitis; this effect was abrogated when both LCs and LangdDCs were ablated together. LC depletion or αGalCer treatment does not affect kidney or lung disease.

Conclusions LCs migrate less, but LangdDCs migrate more, to skin-draining lymph nodes of MRL mice that also have less epidermal γδ T-cells that regulate LC migration via CD40-CD40L interaction. αGalCer that ameliorates dermatitis corrects these defects. Ablation studies suggest that LCs play a protective role, whereas LangdDC play a pathogenic role in lupus dermatitis. Thus, the two skin-DC subsets play opposite, balancing roles in the pathogenesis of lupus dermatitis.