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Immunological changes in atopic dermatitis patients treated with different dosing intervals of dupilumab

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Dupilumab, a fully human IgG4 monoclonal antibody targeting the interleukin-4 receptor alpha (IL4Rα), substantially improves disease severity in patients with atopic dermatitis (AD). While recent daily practice studies have shown that dose reduction of dupilumab is possible in patients with controlled AD, the associated biological effects are currently unknown. Therefore, we studied the dupilumab levels in serum and effects of dupilumab on IL4Rα occupancy, T-cell functions and cytokine production within skin-homing (total CLA+ and CLA+/CCR4+) subpopulations in patients with AD who were treated with 300mg dupilumab subcutaneously every 2 weeks (Q2W), every 4 weeks (Q4W) and every 6 weeks (Q6W). As the time between injections increased, the dupilumab levels in serum and the amount of bound dupilumab (anti-IgG4) decreased. During treatment with 300mg dupilumab Q2W and Q4W, the IL4Rα was completely occupied regardless of the number of days between the injection of dupilumab and blood draw. Simultaneously, the Eczema Area and Severity Index (EASI) score remained stably low and IgE levels declined. During treatment with 300mg dupilumab Q6W, however, the IL4Rα became available again and the percentage of proliferating (Ki67+) and Th2 cytokine producing skin-homing CD4+ T-cells increased compared to dupilumab Q4W. This was also reflected in the clinical parameters with a slightly rising EASI score. In summary, 300mg dupilumab Q2W and Q4W stably inhibited IL4Rα with similar immunological and clinical effects. The biological tipping point for patients with AD who are treated with dupilumab seems to occur at the transition from 300mg dupilumab Q4W to 300mg dupilumab Q6W.

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Targeting pathogenic MICA-NKG2D interactions by statins: A novel adjunct treatment strategy for alopecia areata management?

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JAK inhibitors (JAKi), a major advance in AA therapy may not restore hair follicle's (HF) physiological immune privilege (IP). Yet, HF IP collapse is required for AA to develop, while HFs with intact/restored IP are relatively AA-resistant. Therefore, additional therapeutics are needed that target pathogenic pathways in AA that are unaffected by JAKi. Namely, NKG2D on T and NK cells is activated by the epithelial distress signal, MICA (e.g., by "stressed" HFs), leading to HF IP collapse-inducing IFNg secretion by T/NK cells. Statins may up-regulate expression/activity of the metalloproteinases, ADAM10 and 17, which enhance MICA shedding and may suppress pathogenic MICA-NKG2D interactions. To test this hypothesis in an AA context, we have co-cultured human outer root sheath keratinocytes (ORS-KCs) with human Vd1$^+$T Cells (TCs). These transitional immunity TCs are increased in both lesional and non-lesional AA skin, secrete IFNg via the above MICA-NKG2D mechanism, and attack "stressed" HFs in an AA-like manner *ex vivo*. ORS-KCs were pre-treated with vehicle, IFNg or H_2O_2 to imitate HF IP collapse conditions and oxidative damage-induced HF stress. After 24h, an equal number of human dermal Vd1$^+$TCs was added for 48h, followed by FACS analysis. This showed that Vd1$^+$TCs become activated (increased CD69$^+$/NKG2D$^+$ expression) and up-regulate cytotoxic proteins (perforin, granzyme B) when co-cultured with INFg or H_2O_2-treated ORS-KC. Moreover, MICA expression by ORS-KCs is up-regulated after oxidative stress, which can be counteracted by lovastatin treatment *in vitro*. Lovastatin also up-regulates both ADAM10 and 17 on ORS-KCs, which should enhance MICA shedding. This pilot study supports to systematically re-examine well-tolerated statins as a potential adjunct AA therapeutic in future AA management that down-regulates pathogenic, "innate" NKG2D-MICA interactions.

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Memory B cells of atopic individuals preferentially express IL-31RA: a putative role of IL-31 in B cell biology

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The novel cytokine IL-31 and its receptor have been shown to play a central role in bridging the immune system with neurons, epithelial surfaces and connective tissues. Although increasing evidence has demonstrated their role in inflammation, their involvement in the expansion of distinct leukocyte subsets is not fully understood. Detailed analyses of *Il31*-transgenic (Eμ-Lck) mice indicated that next to the induction of pruritus and skin lesions, mice also presented enlarged lymph nodes with increased B cell frequency. Therefore, we aimed at characterizing the role of IL-31 signaling in B cell biology. Using flow cytometry, the frequency of total B cells and specific B cell subsets was assessed in peripheral lymph nodes and bone marrow of *Il31*-transgenic (Eμ-Lck) mice and wild-type controls. Moreover, IL-31RA expression was analyzed on human B cell subsets from peripheral blood of healthy and atopic dermatitis donors. We observed a significantly increased frequency of CD19$^+$ B cells in bone marrow and peripheral lymph nodes in *Il31*-transgenic mice. Detailed analyses of B cell subsets indicated that *Il31*-transgenic mice showed a higher frequency of plasmablasts in peripheral lymph nodes. In human, memory B cells represented the major population expressing IL-31RA within the B cell compartment. Furthermore, atopic dermatitis patients with elevated IgE levels (>1000 kU/l) showed a higher IL-31RA expression on memory B cells compared to healthy donors. This is of particular interest since DOCK8 acts as a negative regulator of IL-31. DOCK8 deficiency-related hyper-IgE syndrome presents with elevated serum IgE levels and severe atopic dermatitis with increased IL-31 expression. Taken together, these findings point to a novel role of IL-31 in B cell biology and an atopic IgE-producing phenotype.

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A role for FcγRIIB in systemic sclerosis

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Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by excessive fibrosis of the skin and internal organ. FcγRIIB, a low-affinity receptor for the Fc fragment of IgG, is expressed on the surface of several leukocyte subsets and functions in negative feedback pathways to down-regulate B cell antigen receptor signaling. To elucidate the role of FcγRIIB in the development of fibrosis, a murine bleomycin (BLM)-induced scleroderma model was examined in mice lacking FcγRIIB (FcγRIIB$^{-/-}$ mice). FcγRIIB expression on B cells of patients with SSc and healthy controls was also examined. The severity of fibrosis in the skin and lungs was significantly greater in BLM-treated FcγRIIB$^{-/-}$ mice than in BLM-treated wild-type (WT) mice. In the skin of BLM-treated mice, the numbers of CD8$^+$ T cells, F4/80$^+$ macrophages, MPO$^+$ neutrophils, NK1.1$^+$NK cells, and B220$^+$ B cells were significantly higher in FcγRIIB$^{-/-}$ mice than in WT mice. The expression of TNF-α and IL-1β was significantly higher in FcγRIIB$^{-/-}$ mice than in WT mice as was the expression of ICAM-1, CXCL2, and CCL3 in the affected skin. Adoptive transfer of splenic leukocytes from FcγRIIB$^{-/-}$ mice into WT mice resulted in exacerbated skin and lung fibrosis compared with WT mice without adoptive transfer. These results indicate that FcγRIIB plays an inhibitory role in skin and lung fibrosis. On the other hand, the expression levels of FcγRIIB on SSc naïve and DN memory B cells were significantly increased compared to healthy controls. Increased FcγRIIB expression levels on double negative memory B cells were associated with presence of interstitial lung disease. Thus, SSc B cells may exhibit compensatory elevation in the expression levels of FcγRIIB in order to suppress the abnormal activation of B cells.

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Possible plasticity of cytotoxic resident memory T cells in fixed drug eruption

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Fixed drug eruption (FDE) repeats in the same sites and the lesions increase by repetitive exposure to the causative drug. Although FDE is regarded to be mediated by skin resident memory T cells (TRM), which are sessile and do not recirculate from skin, the clinical characteristics of FDE cannot be fully explained by the known functions of TRM in human. In order to elucidate the contribution of TRM in the FDE manifestation, skin T cells isolated from lesions of total five recurrent and four cured FDE subjects, and total seven controls from surgical discards were analyzed for the expression of cell-surface molecules representing the phenotypes of TRM and central memory T cells (TCM) by flow cytometry. Then, recurrent FDE lesions turned out to be enriched with T cells bearing the mixed phenotype of TCM and cytotoxic TRM with CD49a expression (% TCM in CD8: 22.54 ± 10.59 % in recurrent FDE, 1.14 ± 1.21 % in cured FDE, 2.70 ± 2.91 % in control, $p < 0.05$ between recurrent and cured FDE; % CD49a in CD8: 94.51 ± 4.52 % in recurrent FDE, 84.99 ± 4.29 % in cured FDE, 52.74 ± 25.57 % in control, $p < 0.05$ between recurrent FDE and control). In two recurrent FDE subjects, the isolated lesional CD49a$^+$ cytotoxic TRM, lesional CD49a$^-$ CCR7$^+$ TCM, blood TCM, and blood effector memory T cells were analyzed for the commonality of T-cell receptor (TCR) repertoire by high-throughput sequencing analysis. Actively proliferating lesional cytotoxic TRM and lesional TCM shared the same TCR repertoire, with 23 TCR clones overlapped between 186 lesional cytotoxic TRM clones and 193 lesional TCM clones in FDE 1, and 23 clones overlapped between 400 lesional cytotoxic TRM clones and 269 lesional TCM clones in FDE 2. However, these common TCR clones were not found from blood TCM fraction in both subjects. Our results thus imply the differentiation direction from lesional cytotoxic TRM to lesional TCM phenotype in human, and this plasticity may explain the expanding disease nature of FDE.

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The Role of FasL-dependent Signaling in the Pathogenesis of Pemphigus Vulgaris

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Pemphigus vulgaris is a chronic, life-altering bullous autoimmune disease with production of antibodies against the desmosomal proteins Desmoglein 3 and 1 (Dsg) causing acantholysis. Ubiquitous antibody deposition but discontinuous disease manifestation indicate the possible role of cofactors. In this study, we focus on FasL as a putative cofactor in PV-related acantholysis. To study cell-death dependent processes during PV, we used the spontaneously immortalized keratinocyte cell line HaCaT stimulated with AK23 (anti-Dsg3 ab) together with FasL as a cofactor. To this aim we 1. tested the stability of monolayers in the dispase-based keratinocyte-dissociation assay, 2. studied the cleavage status of various proteins by Western Blot and 3. investigated cell viability and cell death via Crystal Violet and FACS. FasL is an activator of the extrinsic apoptotic pathway. In our experiments, we first defined a sublethal concentration of FasL that was sufficient to activate the caspase cascade but did not yet lead to acantholysis in vitro. Costimulation with FasL and AK23 resulted in an increased fragmentation of the monolayers compared to stimulation with AK23 alone. Importantly, treatment of the monolayers with AK23 alone for 4h led to a partial cleavage of caspase 8 (initiator caspase), while the following apoptotic caspase cascade was not activated. Even after prolonged incubation with AK23, there was no increase in cell death. In line with these results, the pan-caspase inhibitor zVAD-fmk could not prevent from AK23-induced acantholysis. This was further confirmed in HaCaT cells overexpressing cFLIP short in which caspase 8 activation is blocked. These data suggest that although AK23-induced caspase 8 activation is not relevant for early acantholytic processes, apoptotic caspase cascade-inducing cofactors may have an impact on the pathogenesis of pemphigus vulgaris. This is of great interest due to possible interactions with inflammatory caspases and other signaling pathways in context of pathophysiology and therapeutic approaches.