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The tryptophan metabolism enzyme, L-kynureninase, is a novel inflammatory factor in psoriasis and other inflammatory diseases

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L-kynureninase (KYNU), an enzyme in the tryptophan metabolism pathway, is habitually found as a differentially expressed gene (DEG) in psoriasis. Although many DEGs have known function in regards to psoriasis and inflammation, KYNU has been completely unexplored in both psoriasis and within general immunology. Here we show that psoriatic lesional skin has significantly increased KYNU mRNA, and is infiltrated by large numbers of $KYNU^+$ cells, as determined by qRT-PCR and immunohistochemistry, respectively. The identity of $KYNU^+$ cells was elucidated using both two-color immunofluorescence and qRT-PCR. Although KYNU is expressed in several cell lineages, myeloid subsets were found to express 100-fold more KYNU compared to all other cell types, suggesting a likely immunomodulatory role for this enzyme. To determine how KYNU may contribute to inflammation, primary human cutaneous cells were cultured with specific tryptophan metabolites, and modulation of gene expression was assessed. Interestingly, metabolites downstream of KYNU induced significant upregulation of several inflammatory genes (including IL-20, IL-8, CCL2, CXCL1, VCAM, ICAM, and E-selectin), with the metabolite directly produced by KYNU (3-hydroxyanthranilic acid) inducing the highest amount of inflammatory gene upregulation. Conversely, metabolites upstream of KYNU (such as the AhR-ligand, kynurenine) had minimal ability to induce and even suppressed expression of some inflammatory genes. Therefore, KYNU may be a pivotal switch in tryptophan metabolism and dictate an inflammatory outcome. By mining gene array data, we found many other human diseases, including atopic dermatitis and lupus, also contain elevated KYNU, suggesting a role of this enzyme not only in psoriasis, but also in general cutaneous inflammation.

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Predictors of skin disease outcome in rituximab-treated refractory dermatomyositis patients

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The purpose of the study was to identify clinical and laboratory predictors of skin disease outcome in patients with refractory dermatomyositis (DM) treated with rituximab. We analyzed data for 116 DM patients (68 with adult DM and 48 with juvenile DM) in the Rituximab in Myositis trial. Skin disease outcome was defined as the absolute change from baseline to 24 weeks in the Cutaneous Disease Activity visual analog scale (VAS) score where a negative change indicated improvement and a positive change indicated worsening. We analyzed the association of the following baseline variables with skin disease outcome: demographics, dermatomyositis subtype, clinical and laboratory parameters, myositis autoantibodies (antisynthetase, anti–Mi-2, other autoantibodies, and no autoantibodies), and interferon (IFN)-regulated chemokines. Multivariable linear regression models were developed using stepwise variable selection methods. A multivariable model to predict skin disease outcome was built with good predictive ability (adjusted R^2=0.33). The model included three factors at baseline: Cutaneous Disease Activity VAS score (b [regression coefficient] = -0.48, P < 0.01), Constitutional Disease Activity VAS score (b = 0.20, P 0.02), and anti–Mi-2 status (b = -5.71, P < 0.01). This indicates that participants with higher Cutaneous Disease Activity VAS scores at baseline were more likely to improve in their Cutaneous Disease Activity VAS scores from baseline to 24 weeks, participants with higher Constitutional Disease Activity VAS scores at baseline were more likely to worsen, and participants with positive anti–Mi-2 status at baseline were more likely to improve. This model could be clinically useful to optimize treatment selection in DM patients with recalcitrant skin disease.

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TNF-α antagonist induced cutaneous lupus

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TNF-α antagonist-induced lupus-like syndrome (TAILS) is a rare entity characterized by exposure to a TNF-α inhibitor, ≥1 new symptom of lupus erythematosus (LE), no prior history of LE, and symptom resolution upon medication discontinuation. It is more common in women in their fifties and among infliximab users. Cutaneous involvement can include malar rash, photosensitivity, subacute cutaneous LE (SCLE), and discoid lupus-type lesions. We present a case of a 57-year-old female receiving infliximab infusions for rheumatoid arthritis (RA) who presented with an 8-month history of a progressively worsening facial eruption. Physical exam revealed periorbital edema, with erythematous papules and plaques on the cheeks and forehead. Review of systems was otherwise negative. The patient noted the eruption initially coincided with a new-onset pneumonia. Punch biopsies revealed superficial and deep perivascular and interstitial mixed-cell inflammatory infiltrates comprised predominately of lymphocytes and histiocytes, with occasional neutrophils and increased dermal mucin, consistent with a connective tissue disease process. Labs revealed ANA of 1:640 (homogenous pattern) and elevated dsDNA. The facial eruption improved upon discontinuation of infliximab and initiation of chloroquine, and autoantibodies normalized. Thereafter, her RA was successfully managed by abatacept without lesion recurrence. While the pathogenesis of TAILS is not entirely understood, one proposed mechanism is that relative immunosuppression encourages increased infection rates and activation of polyclonal B lymphocytes, which stimulates autoantibody production.[1] This case may be a clinical illustration of this mechanism, as the patient developed a rash and autoantibodies consistent with cutaneous LE while on a TNF-α inhibitor and soon after infection, with resolution upon antimalarial therapy and discontinuation of infliximab.

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Dermal vascular changes in the C3H/HeJ alopecia areata mouse model

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Mouse models with various types of inflammatory skin disease are often accompanied by increased angiogenesis as is the case for the flaky skin ($Ttc7^{fsn}$) and chronic proliferative dermatitis ($Sharpin^{cpdm}$; $Sharpin^{cpdm\text{-}Derm}$) models. The C3H/HeJ inbred strain spontaneously develops alopecia areata (AA), a cell mediated autoimmune disorder which can be controllably expanded using full thickness skin grafts to young unaffected mice. This provides a reproducible and progressive model for AA in which the vascularization of the skin can be examined. Mice receiving skin grafts from AA or sham surgery were evaluated at 5, 10, 15, and 20 weeks after engraftment. Blood vessels were found to be slightly more numerous and dilated compared to controls by immunohistochemistry. This was more pronounced using antibodies directed against smooth muscle actin isoforms than CD31. Lymphatics are often overlooked as they are small slit-like dermal structures above the angle of the hair follicles. These lymphatics are often missed as they resemble artifactual separation of collagen bundles with some fixatives. Lymphatics are easily detected using LYVE1 by immunohistochemistry to label their endothelial cells. Using LYVE1 there were no changes in distribution or numbers of lymphatics although they were more prominent (dilated) in the mice with alopecia areata. Lyve1 transcripts were not significantly upregulated except at 10 weeks after skin grafting when clinical signs of AA first become apparent. Other genes involved with vascular growth and dilation were dysregulated. These findings emphasize aspects of AA not commonly considered in the evaluation of human and other species affected.

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Langerhans cells promote the development of imiquimod-induced psoriasis-like dermatitis by producing IL-23

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Psoriasis is an autoinflammatory skin disease that involves interplay among several types of cells, including keratinocytes, T cells anddendritic cells (DCs); however, the role ofLangerhans cells (LCs),specificsubset of DCsin epidermis, in the pathogenesis of psoriasis is not clear. In the present-study, we treated C57BL/6 mice with imiquimod (IMQ)over 5 consecutive days to induce psoriatic-dermatitis, and responses of LCs were analyzed subsequently. The number of LCs in the epidermis increased after IMQ treatment, and theproliferation level of LCs after IMQ treatment was higherthan that of LCs under steady state. LCs showed a phenotype of maturation by expressing higher level of MHC-II, CD86, CD80, CD40 after IMQ treatment. There was also elevated level of langerin on LCs after IMQ stimulation. LCs expressed increased level of IL-23, as indicated by intracellular staining and RT-PCR for the sorted LCs from the epidermis of IMQ-treated skin. Migration of LCs was accelerated after IMQ treatment, and there was increased number of LCs in the draining lymph node, which showed the phenotype of maturation by expressing higher level of CD86, CD80 and CD40. In regional lymph node, LCs also expressed higher level of PD-L1 and PD-L2 after IMQ treatment.In addition, LCswere sortedfrom naive C57BL/6 mice and were cultured for 24h with IMQ, and significantly increasedexpression ofIL-23was observed.Taken together, ourstudyshowedthat LCs promote the development of IMQ-induced psoriasis-like dermatitis by producing IL-23, and targeting LCs might be a future strategy for the treatment of psoriasis.

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Essential requirement for IRF7 in the production of autoantibodies in murine lupus

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Systemic lupus erythematosus (SLE) is considered to be the prototypic systemic autoimmune disease characterized by the production of autoantibodies against nuclear components. Recent genetic studies of SLE patients have revealed that interferon regulatory factor (IRF) 7 gene polymorphisms are associated with an increased risk of SLE but the precise role of IRF7 in SLE development is not fully understood. IRF7 is a member of the IRF family of transcription factors that induce transcription of IFN-α and the expression of interferon (IFN)-stimulated genes (ISGs). To investigate the role of IRF7 in the pathogenesis of SLE, a murine model of SLE induced by 2,6,10,14-tetramethylpentadecane (TMPD) was used. IRF7 deficient mice and wild-type (WT) control mice were treated intraperitoneally with TMPD and spleens, kidneys and blood were harvested 2 weeks or 10 months later. Proteinuria was detected on dipstick analysis in both WT and IRF7 deficient mice and direct immunofluorescence of the kidneys revealed glomerular IgG deposits in both strains to the similar extent. Interestingly, anti-dsDNA, ssDNA, RNP and Sm autoantibodies were not detected by ELISA in the sera from IRF7 deficient mice but were present in the sera from WT mice. Real-time PCR showed that spleen cells from IRF7 deficient mice expressed substantially lower levels of ISGs as compared with WT mice. However, NF-κB target genes were upregulated in both strains suggesting that type I IFN pathway was critical in autoantibody production but NF-κB activation was sufficient for development of glomerulonephritis. These results demonstrate a specific requirement for IRF7 in autoantibody production against the DNA- and RNA-containing antigens and autoantibody production and lupus nephritis are controlled by separate mechanisms in this model.

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Skin-homing and systemic T-cell subsets show higher activation in atopic dermatitis versus psoriasis

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Atopic dermatitis (AD) and psoriasis are characterized by T-cell infiltration in skin lesions, but their comparable systemic T-cell activation is unclear. We compared T-cell activation and cytokine producing cell-frequency in blood of adult AD and psoriasis patients using flow-cytometry. We measured cytokines, T-regulatory cells (Tregs) and T-cell activation markers in central and effector memory (Tcm and Tem) skin homing/cutaneous lymphocyte antigen (CLA^+) and CLA^- subsets from 24 psoriasis patients, 35 AD patients and 13 controls. Early (CD69), mid (ICOS) and late (HLA-DR) activation markers were quantified in Tcm ($CCR7^+CD45RO^+$) and Tem ($CCR7^-CD45RO^+$) populations. AD showed higher frequency of CLA^+ "polar" T-cell subsets ($p<0.0001$). In both diseases, CLA^+ T-cells were significantly more activated compared to respective CLA^- subsets ($p<0.01$), suggesting their prominent role in inflammatory skin diseases. AD demonstrated higher levels of ICOS/HLA-DR activation in circulating CLA^+ and CLA^- memory subsets ($p<0.01$). CD69 was the only activation marker that was higher in psoriasis ($p=0.001$), whereas ICOS expression was significantly higher in AD ($p<0.0001$), compatible with their respective roles in Th17 and Th2 responses. Significant correlations with SCORAD were observed in AD, particularly striking for ICOS ($r=0.5$, $p<0.01$). Higher $CD25^+CD127^-CCR4^+CLA^+$ Tregs were found in AD, correlating with SCORAD and IgE. Compared with psoriasis, AD is characterized by increased polar differentiation of Tcm/Tem subsets, with higher, and persistent activation particularly within skin homing subsets. Higher systemic activation in AD might reflect the wide abnormalities seen in non-lesional skin in AD compared to psoriasis, emphasizing the large need for systemic treatment approaches for severe AD patients.

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Ajulemic acid, a novel cannabinoid, suppresses the secretion of tumor necrosis factor alpha and interferon alpha from the peripheral blood mononuclear cells of dermatomyositis patients *in vitro*

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Ajulemic acid (AJA) is a synthetic, non-psychoactive analog of a metabolite of tetrahydrocannabinol with anti-inflammatory properties potentially useful for treating autoimmune skin disease. Experiments presented here quantified the capacity of AJA to suppress the secretion of tumor necrosis factor alpha (TNFα) and interferon alpha (IFN-α), cytokines implicated in the pathogenesis of dermatomyositis (DM), from peripheral blood mononuclear cells (PBMCs) isolated from patients with DM. The PBMCs were incubated with 3, 10 and 15 μM AJA with or without stimulation by lipopolysaccharide (LPS) in the case of TNFα or CpG oligodeoxynucleotides (CPG) in the case of IFN-α. ELISA was used to quantify the PBMCs' secretion of TNFα and IFN-α. Unstimulated PBMCs incubated with 3 (n=13), 10 (n=16) and 15 (n=13) μM AJA reduced secretion of TNFα by a median (interquartile range) of 27.1% (-55.4% – 76.5%), 23.4% (-51.6% – 76.6%) and 83.9% (29.9% – 96.1%), respectively, compared to unstimulated PBMCs without AJA incubation. Unstimulated PBMCs produced insignificant levels of IFN-α. LPS-stimulated PBMCs that were incubated with 3 (n=16), 10 (n=16) and 15 (n=15) μM AJA reduced secretion of TNFα by a median (interquartile range) of -48.3% (a net increase; -101.1% – 13.9%), 75.9% (29.8% – 96.7%) and 95.4% (68.8% – 99.5%), respectively, compared to LPS-stimulated PBMCs that were not incubated with AJA. The median (interquartile range) quantity of IFN-α secreted from CPG-stimulated PBMCs incubated with 3 (n=7), 10 (n=8) and 15 (n=7) μM AJA compared to those with no AJA incubation decreased by 93.4% (82.8% – 99.7%), 99.6% (98.3% – 100.0%) and 100.0% (99.8% – 100.0%), respectively. AJA suppressed secretion of TNFα and IFN-α from the stimulated PBMCs of DM patients in vitro. Thus, AJA may offer a novel, and potentially less toxic, treatment for DM.

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Peripheral blood gene expression identifies systemic pathways and processes in chronic cutaneous lupus erythematosus (CCLE)

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Lupus erythematosus (LE) is an autoimmune systemic disease with confounding etiology and pathogenesis. Cutaneous lesions develop in 80% of LE patients at some point and 10-40% patients with cutaneous LE (CLE) show transitions to systemic LE (SLE). Major gaps remain regarding pathogenetic mechanisms underlying the development of cutaneous lesions in lupus. As systemic changes are likely to underlie skin specific manifestation, we analyzed global gene expression by microarray in peripheral blood in a cohort of CCLE patients and healthy controls. Unbiased clustering analyses identifies a "disease" based signature that distinctly separates patients from healthy controls. Functional annotation of CCLE-blood differentially expressed genes (DEGs) using DAVID and Metacore highlight enrichment of a dysregulated immune response with prominent Type I interferon association as well as apoptosis and cellular breakdown processes. There is a 26% overlap of the CCLE blood and lesional skin transcriptional profile from a previous analysis by our group. Two of the four transcriptional "hot spots" (chromosomal regions harboring statistically increased numbers of CCLE blood DEGs) overlap with previously identified CCLE skin transcriptionally active regions. These "hot spots" offer prioritized loci for downstream fine mapping studies in the search for CCLE specific susceptibility loci. Overall, these data provide further insights regarding the molecular genetic basis of disease heterogeneity in lupus.

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Serum levels of soluble PD-1 and PD-L2 correlate with disease severity in systemic sclerosis

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Purpose: The programmed death-1 (PD-1) which mainly expresses on T cells and its ligand, PD-L1 and PD-L2, show a co-inhibitory effect to T cells, resulting in prevention of excessive immune injury. While PD-L1 expresses on both hematopoietic and nonhemopoietic cells, PD-L2 expression is thought to be restricted to antigen-presenting cells (APCs), including B cells. PD-1/PD-Ls has two forms of expression, a membrane bound form and a soluble form. Many studies have been shown that soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) can promote T cell responses through blocking PD-1/PD-Ls pathway. However, there is no report about the biological function of sPD-L2. Systemic sclerosis (SSc) is generally regarded as an autoimmune disorder because of the presence of autoantibodies. Although the pathogenesis of SSc remains unclear, previous studies have suggested that B cells play an important role in disease development and progression. In this study, we focused on the serum levels of sPD-1 and sPD-L2 in SSc to investigate the relationship of PD-1/PD-L2 and disease severity. *Methods*: Serum levels of sPD-1 and sPD-L2 were examined in SSc (n=94) and healthy controls (n=25) by enzyme-linked immunosorbent assay. We also examined sPD-1 and sPD-L2 levels in bleomycin-induced SSc model mice. *Results*: Serun levels of sPD-1 and sPD-L2 in SSc were significantly higher than in healthy controls ($p<0.01$). SSc patients with elevated serum levels of sPD-1 and sPD-L2 had significantly higher frequency of diffuse cutaneous SSc and more frequent involvement of pitting scar/ulcer and pulmonary fibrosis than those with normal levels ($p<0.05$). Serum levels of sPD-1 and sPD-L2 significantly correlated positively with modified Rodnan total skin thickness score, serum levels of IgG, anti-topoisomerase I antibodies, and C-reactive protein and correlated inversely with %VC and %DLco. In bleomycin-induced SSc model mice, serum sPD-1 and sPD-L2 levels were higher than in control mice ($p<0.01$). *Conclusion*: These results suggest that elevation of serum levels of sPD-1 and sPD-L2 are associated with the disease severity.

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Desmoglein 3 chimeric autoantibody receptor T cells: A novel strategy for immunotherapy of pemphigus vulgaris

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Pemphigus vulgaris (PV) is a potentially fatal blistering skin disease caused by autoantibodies (autoAbs) to desmoglein (Dsg) 3. Therapy for PV relies on general immunosuppression, risking severe infection with chronic use. Recently, chimeric antigen receptor (CAR) T cells have revolutionized cancer immunotherapy. CAR T cells kill antigen-expressing cancer cells and produce memory CAR T cells, leading to durable remission of even late-stage cancers. To evaluate this potent approach for PV, we engineered a series of Dsg3 chimeric autoAb receptors (CAARs), consisting of various Dsg3 extracellular (EC) domains fused to either T or NK receptor cytoplasmic signaling domains. In chromium release assays, primary human T cells expressing Dsg3 CAARs specifically kill target cells expressing surface anti-Dsg3 EC1, EC2, or EC3 autoAbs, with the most broad and potent cytolysis by the Dsg3 EC1-4 CAAR using the T cell CD137CD3ζ signaling domain ($p=0.0001$). Off-target toxicity was not observed, as Dsg3 CAAR T cells do not kill $Fc\text{-}receptor^+$ cells coated with Dsg3 autoAbs or human keratinocytes whose desmosomal cadherins might interact with the Dsg3 CAAR. Live imaging TIRF microscopy reveals that autoAb binding by the Dsg3 CAAR forms supramolecular activation complexes, with segregation of the CAAR away from CD45, a regulator of T cell receptor activation. In a preclinical PV NSG mouse model, bioluminescent AK23 (anti-EC1 surface IgG^+) hybridoma cells were efficiently killed by Dsg3 CAAR ($p=0.0015$), resulting in prolonged survival of CAAR-treated versus control mice ($p=0.016$) without skin toxicity. CAAR T cells represent a novel strategy for PV therapy that specifically targets autoantigen-specific B cells and could readily be applied to other autoAb-mediated diseases.

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Comparison of anti-desmoglein B cell repertoire and anti-desmoglein antibody repertoire in pemphigus patients

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Autoantibodies against desmoglein 1 (Dsg1) or Dsg3 cause skin blistering in pemphigus foliaceus (PF) and vulgaris (PV) respectively. Although anti-Dsg B cell repertoires have been characterized by antibody phage display (APD), heterohybridoma, or EBV immortalization techniques, these methods only show potential antibody coding sequences but do not indicate which antibodies are actually secreted (as opposed to being, for example, memory B cell receptors) or the relative amount of each antibody in serum. Here, we applied high resolution tandem mass spectrometry (MS) on Dsg-affinity purified antibodies to characterize circulating autoantibodies in a PF and a PV patient. A bioinformatics algorithm with strict filters was used to identify the VH-CDR3 peptide sequences which are the signatures of clonal antibodies. In the PF patient we found 4 APD anti-Dsg1 clones, of which only 1 was pathogenic when produced recombinantly and injected into normal human skin. By MS we found only one antibody clone whose VH-CDR3 matched that of the pathogenic APD clone, but none that matched the others. In similar studies with a PV patient we found 7 anti-Dsg3 APD clones, of which only 2 were identified as circulating antibodies by MS. To determine if there were other circulating antibodies not identified by APD clones, we used next generation sequencing of the total IgG mRNA repertoire from blood B cells from the PV patient as a reference database for MS. With this database we identified 7 Dsg3-specific antibodies with unique CDR3s, of which 5 were not found by APD. However, MS indicated one major clonal antibody (about 5 times the amount of the 2nd most abundant clone identified) which was also found by APD. These results indicate that the B cell repertoire and the antibody repertoire in pemphigus are both oligoclonal but not completely overlapped. There is a clonally dominant serological autoimmune response in pemphigus.