O43 Interferon kappa is required for regulation of baseline type I interferon responses in keratinocytes and is dysregulated in cutaneous lupus

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Interferon kappa (IFNK) responses could be transferred to fibroblasts by adding supernatants from KCs, and reversal of disease in mice. Based on these data and clinical observations, we propose that IFNK may be targeted to create a durable treatment response. Notably, cultured KCs from normal CLE skin showed increased baseline pSTAT1 and type I IFN activity in epidermis of active CLE, along with pSTAT1 and pSTAT2 activation. Knock-out (KO) of IFNK in KCs via CRISPR/Cas9 abolished KC IFN secretion (< 0.001), baseline type I IFN gene expression, and basal pSTAT1 and 2 activation. Similarly, KO of TYK2, a signal mediator downstream of type I IFN receptors, abolished baseline type I IFN gene expression in KCs (p = 0.016-0.001), suppressed IFNkr mRNA expression (p = 0.001-0.005) and represented pSTAT1 and pSTAT2 (p < 0.05) and (p < 0.001). While TYK2 KO abolished responses to exogenous IFN, KO was not observed across all concentrations (1, 5, 50ng/ml). IFNKO delayed and minimized KC responses to exogenous IFN-α (p < 0.001). Cutaneous lupus (CLE) is characterized by type I IFN responses, which we found (MAX, R2K, p < 0.001 for all). Of the type I IFN family, IFNκ was the most increased in CLE (p = 0.001, n = 90), and confirmed by IFN showing prominent IFN expression in epidermis of active CLE, along with pSTAT1 and pSTAT2 activation. Notably, cultured KCs from normal CLE skin showed increased baseline pSTAT1 and pSTAT2 activation and higher IFN production compared to KCs from healthy controls. CLE KCs had heightened responses to exogenous IFN that could be suppressed to healthy control levels by addition of anti-IFNκ antibody (MAX, p < 0.05). Our data identifies IFNκ as essential for IFN responses in KCs and helps to explain the clinical observation of why blockade of the type I IFN receptor is more effective than targeting IFNκ in CLE.

O44 Exploring a possible role for Indoleamine 2,3-deoxigenase (IDO) and the Host Immune Response in Infantile Hemangioma

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Indoleamine 2,3-deoxigenase (IDO) is a cytochrome p450 enzyme that deaminoetylizes tryptophan, producing kynurenine. IDO expression has been described in many human malignancies, autoimmune diseases and in animal models of inflammation. IDO has been shown to inhibit Th17 cells which exhibit a fully differentiated signature after 24h, Tr1 cells have longer and stable CLP state, which is defined by a fully differentiated signature in cytokines in cutaneous autoimmune diseases and GVHD. Unlike the well-described Foxp3+ regulatory T cells, the molecular mechanisms regulating Foxp3+ T cell development and IL-10 production are largely undefined. Using single cell RNASeq, NanoString codets, and microarrays, we defined a transcriptional profile for Tr1 cell differentiation, both in vitro and in vivo models of epithelial autoimmune disease. To identify regulators critical to Tr1 cell-specific differentiation, we compared the dynamic regulatory network controlling Th17 cells that precede Tr1 cells (both in vitro and in vivo), and Th1 cells that arise from a naive-like state to Tr1; early, intermediate, and late. Moreover, unlike Th17 cells which exhibit a fully differentiated signature after 24h, Th1 cells have longer and more distinct waves of expression until complete differentiation. Our analysis also points to transcriptional similarity between the two cell types, mainly in early time points, associated with activation, and very late time points. Functional perturbation of candidate regulators allowed us to generate a network of core transcription factors sufficient for Tr1 cell development and IL-10 production. Insight into Tr1 cell development and IL-10 production may provide an avenue for therapeutic intervention in cutaneous autoimmune disorders.

O45 Dynamic regulatory network controlling Tr1 cell development in epithelial tissue inflammation

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Tr1 cells are a class of regulatory T cells that produce IL-10 and suppress tissue inflammation in cutaneous autoimmune diseases and GVHD. Unlike the well-described Foxp3+ regulatory cells, the molecular mechanisms regulating Foxp3+ T cell development and IL-10 production are largely undefined. Using single cell RNASeq, NanoString codets, and microarrays, we defined a transcriptional profile for Tr1 cell differentiation, both in vitro and in vivo models of epithelial autoimmune disease. To identify regulators critical to Tr1 cell-specific differentiation, we compared the dynamic regulatory network controlling Th17 cells that precede Tr1 cells (both in vitro and in vivo), and Th1 cells that arise from a naive-like state to Tr1; early, intermediate, and late. Moreover, unlike Th17 cells which exhibit a fully differentiated signature after 24h, Th1 cells have longer and more distinct waves of expression until complete differentiation. Our analysis also points to transcriptional similarity between the two cell types, mainly in early time points, associated with activation, and very late time points. Functional perturbation of candidate regulators allowed us to generate a network of core transcription factors sufficient for Tr1 cell development and IL-10 production. Insight into Tr1 cell development and IL-10 production may provide an avenue for therapeutic intervention in cutaneous autoimmune disorders.

O46 Characterization of a conformational epitope on the EC1 domain of desmoglein 1 recognized by IgG4 autoantibodies from Fogo Selvagem

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Fogo Selvagem (FS) is mediated by pathogenic IgG4 restricted autoantibodies against Desmoglein-1 (DG1), which do not react with Dsg1 or Dsg4. Double purified FS anti-Dsg4 IgG4 (affinity purified on llama anti-human IgG4-Agarose and a human Dsg1-ectodomain-Agarose, which yield highly pathogenic IgG4 in the mouse model. Hybrid Dsg1 molecules bearing the EC1 domain of Dsg4 no longer bind FS IgG4. Moreover, Dsg4 bearing the EC1 domain of Dsg1 gain reactivity with FS IgG4 and are able to affinity-purify FS IgG4 autoantibodies that are also pathogenic in the mouse model, further suggesting that pathogenic epitopes are clustered on the EC1 domain of Dsg1. We showed previously that highly purified FS IgG4 from 19 FS sera bind a single peptide on the EC1 domain of Dsg1 using MALDI-MS Epitope Mapping. A 16 amino acid peptide (AENSMGCQDLLRPLL) that overlaps RAL motif of Dsg1 was recognized by 19 IgG4 fractions. Mutations of M131 and Q135 of this peptide reduced the binding (≈50%) of IgG4 autoantibodies to Dsg1 by ELISA. Using molecular modeling we identified two additional residues, distant from the previous peptide, but within the EC1 domain, that are complementary components of the epitope bound by FS IgG4 autoantibodies. Mutations of these additional residues completely ablate the binding of FS IgG4 from 19 FS sera. These results suggest that highly restricted and pathogenic IgG4 autoantibodies from FS patients are exquisitely specific for a conformational epitope on the EC1 domain of Dsg1. This strong FS IgG4 anti-Dsg1 reactivity is specific to this conformational epitope, that partially overlaps the adhesive site of Dsg1, may directly interfere with the adhesive function of this molecule and lead to acantholysis.

O47 Vogt-Koyanagi-Harada disease: new insights from genetic studies


Vogt-Koyanagi-Harada disease (VKH) is an autoimmune disease caused by autoimmune-reactive CD8+ T cells that kill melanocytes, resulting in patchy depigmentation. Treatment options are limited, and depigmentation rapidly recurs after cessation of therapy. Notably, cultured KCs from normal CLE skin showed increased baseline pSTAT1 and type I IFN activity in epidermis of active CLE, along with pSTAT1 and pSTAT2 activation. Knock-out (KO) of IFNK in KCs via CRISPR/Cas9 abolished KC IFN secretion (< 0.001), baseline type I IFN gene expression, and basal pSTAT1 and 2 activation. Similarly, KO of TYK2, a signal mediator downstream of type I IFN receptors, abolished baseline type I IFN gene expression in KCs (p = 0.016-0.001), suppressed IFNkr mRNA expression (p = 0.001-0.005) and represented pSTAT1 and pSTAT2 (p < 0.05) and (p < 0.001). While TYK2 KO abolished responses to exogenous IFN, KO was not observed across all concentrations (1, 5, 50ng/ml). IFNKO delayed and minimized KC responses to exogenous IFN-α (p < 0.001). Cutaneous lupus (CLE) is characterized by type I IFN responses, which we found (MAX, R2K, p < 0.001 for all). Of the type I IFN family, IFNκ was the most increased in CLE (p = 0.001, n = 90), and confirmed by IFN showing prominent IFN expression in epidermis of active CLE, along with pSTAT1 and pSTAT2 activation. Notably, cultured KCs from normal CLE skin showed increased baseline pSTAT1 and pSTAT2 activation and higher IFN production compared to KCs from healthy controls. CLE KCs had heightened responses to exogenous IFN that could be suppressed to healthy control levels by addition of anti-IFNκ antibody (MAX, p < 0.05). Our data identifies IFNκ as essential for IFN responses in KCs and helps to explain the clinical observation of why blockade of the type I IFN receptor is more effective than targeting IFNκ in CLE.

O48 Characterization of a conformational epitope on the EC1 domain of desmoglein 1 recognized by IgG4 autoantibodies from Fogo Selvagem

This work was supported by the National Institutes of Health (AI120444 and AI120444). These results suggest that highly restricted and pathogenic IgG4 autoantibodies from FS patients are exquisitely specific for a conformational epitope on the EC1 domain of Dsg1. This strong FS IgG4 anti-Dsg1 reactivity is specific to this conformational epitope, that partially overlaps the adhesive site of Dsg1, may directly interfere with the adhesive function of this molecule and lead to acantholysis.

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