013 Impaired function of ECM1 underlies the pathogenic disorganization of vascular and basement membrane molecules in lichen sclerosus
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Lichen sclerosus (LS) is an acquired inflammatory condition that mainly affects genital skin. EGFR and SMO are associated with vascular and basement matrix maculae plaquing in a substantial number of patients may hold clues in understanding the pathogenetic signature of LS. Indirect immunofluorescence staining on cortical laser scanning microscopy using sera from LS patients (n=23; 1 male and 22 female) displayed that 9 (39.1%) of them exhibit intense immunoreactivity against lower epidermal basement membrane zone sera consistent with the staining pattern of affinity-purified IgG from LS sera and rabbit anti-ECM1 antibody. To address the relationship between in vivo ECM1 dysfunction and molecular events in the dermal matrix, we generated human dermal fibroblasts with siRNA knockdown for ECM1 and analyzed transcription profiles by cDNA microarray. Comparison with siRNA-untreated fibroblasts identified 3,015 differentially expressed genes. Functional annotation assigned that 1,471 upregulated and 1,564 downregulated genes are related to proteins binding DNA/RNA, signal transduction, exosome, and gene regulation. Upon evaluating in vivo localization and proposed function of ECM1 in the skin narrowed to 49 upregulated genes, including COL4A4, lamin A, fibronectin, MMPs, CTGF, PDGFA and its receptor, SMAD, and TGFBR receptor. Real time RT-PCR and ELISA supported the upregulation of panedated genes and corresponding proteins. Laminin 312 and type IV collagen, the representatives upregulated by ECM1 silencing, revealed unique expression pattern on immunohistochemistry using LS skin. Moreover, type VII collagen, which did not satisfy the ECM1-silencing upregulation but showed abnormal expression pattern in the LS skin, border to ECM1 recombinant protein. Impaired ECM1 function may thus cause increased expression and selective disassembly of basement membrane, vascular, and extracellular matrix moleculie, as well as growth factors facilitating fibroblast proliferation, contributing to the LS skin pathology.

014 Inhibitory effect of kaempferol on skin fibrosis in systemic sclerosis by the suppression of oxidative stress
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There is growing evidence that vasculopathy-induced hypoxia and oxidative stress enhance the process of fibrosis in systemic sclerosis (SSc). Kaempferol is a natural flavonoid widely found in various vegetables and fruits, and has been reported to have excellent antioxidant activity. Objective was to elucidate the effect of kaempferol on skin fibrosis and the mechanism of the inhibitory regulation of fibrosis by kaempferol. We assessed the effect of intra-perithecially administered kaempferol on bleomycin-induced dermal fibrosis in mice. The effect of kaempferol on oxidative stress in bleomycin-treated mice and SSC fibroblasts was assessed in vitro and in vivo. We identified several specific genes significantly inhibited in bleomycin-induced dermal fibrosis in mice. The number of CD3+ T cells, and CD66+ macrophages in lesional skin was significantly decreased by kaempferol injections. Kaempferol administration also significantly suppressed the bleomycin-induced oxidative stress signal in OKD4 mice. Additionally, mRNA levels of oxidative stress-associated factors, such as HO-1 and NQO1, as well as inflammatory and pro-fibrotic cytokines, including IL-6, TGF-β and TGFα in sclerotic skin were significantly decreased by kaempferol. Kaempferol also reduced bleomycin-induced TUNEL+ apoptotic cells in the lesional skin of bleomycin-treated mice. Furthermore, the oxidant-induced intracellular accumulation of reactive oxygen species (ROS) in SSC fibroblasts was inhibited by kaempferol treatment. In addition, the oxidant-induced apoptosis of SSC fibroblasts was decreased by kaempferol in vitro. Kaempferol might improve bleomycin-induced fibrosis by reducing oxidative stress in SSC.

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016 Phenotypic Changes of a Monocyte Cell Line depend on the Culture Temperature
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The skin is a unique organ because it is maintained at around 37 °C, the temperature of skin, which is facing directly to external environment, is affected by external climate. Since epidermal Langerhans cells reside in upper most layers of skin, they must be affected with temperature shift. But most of the in vitro experiments are conducted at 37 °C. In this study, therefore, the phenotypic characteristics of Langerhans cells were examined using a model cell line. THP-1 cells. Since the skin surface temperature goes down to 25 °C when people go in to a room at 10 °C, we chose the culture temperature at 29 °C, 31 °C, and regular 37 °C. The proliferation rate declined as temperature went down. The amount of RNA extracted from the same number of cells cultured at lower temperature was smaller than at 37 °C. These results suggest that cellular activity is lower at lower temperature. However, when gene expression was examined, some specific genes were detected at lower temperature, including inhibitor of NFκB, inhibitor of growth family2, B cell anti-translocator gene antiproliferative, while inflammatory genes were expressed at 37 °C, demonstrating that cells are not simply inactive but actively responding to lower temperature. The expression of CD86 examined by flowcytometry was same or a little bit lower at lower temperature compared to 37 °C, but higher after stimulation with diminichlorenzone. These results suggest that epidermal Langerhans cells actively stay quiet at regular physiological condition. These results anyway tell us to consider the culture condition of in vitro experiments when extrapolating to in vivo.

017 Tc2 induction of IL-10-producing plasmablasts during contact hypersensitivity
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B cells include fractions with regulatory function named regulatory B cells (Breg). Various B cell-intriguing signal have been reported as Breg at various disease models. There have been a common characteristics found except for IL-10 production. For the purpose of this study is to identify IL-10-producing Breg in mouse contact hypersensitivity (CHS) model and reveal the mechanism of regulation. CHS was induced in C57BL/6 wild-type mice (WT) and IL-10 knockout mice (IL-10KO) by applying 0.5% 2,4-dinitrochlorobenzene on abdomen for sensitization and 0.25% on the ear for elicitation 7 days after sensitization. IL-10Venus/+ mice were employed to identify IL-10-producing Breg in mouse contact hypersensitivity model and reveal the significance of Breg. Th cell2 induction of IL-10-producing plasmablasts during contact hypersensitivity was much lower in those treated with CS. Our study demonstrated that the concentration of anti-IgE AAbs in sera from patients with higher concentrations of anti-IgE AAbs relative to the cutoff value. The ability of anti-IgE AAbs, but not anti-FcRn AAbs, to induce FcRn crosslinking was significantly higher in CSU patients than in NC subjects (P < 0.0106). In the Japanese population of CSU patients studied, the ability of the anti-IgE AAbs to induce FcRn crosslinking differed significantly between NC subjects and CSU patients, suggesting the involvement of anti-IgE AAbs in the pathogenesis of CSU in the Japanese population.

018 Evolution of autoreactive B and T cells in pemphigus patients with Rituximab or corticosteroida regimen treatment
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Pemphigus is an autoimmune blistering disease mediated by autoantibodies (Abs) directed against desmogleins (Dsg). We recently showed that first line treatment with Rituximab (RTX) was more effective than standard oral corticosteroid (CS) treatment. To understand the immunological mechanisms that mediate the long-lasting clinical remission (CR) after RTX treatment, we analyzed the phenotype and antigen specificity of B cells and T cells helper cells (ThF) by flow cytometry and the number of Dsg Abs Secreting Cell (ASC) by ELISPOT. At Baseline, Dsg 1 and 3 Abs were detected in a frequency of 0.1 to 0.6% of total B cells and were in the 10 to 50% of Dsg 1 switched memory B cells. Dsg 3 Abs were detected at a frequency of 0.8% of total ASC for Dsg 1 and 0.12% for Dsg 3. The CS treatment did not influence the frequency nor the phenotype of Dsg 1 B cells and Dsg 3 ASC, which were detected even in patients in CR. In contrast, RTX induced a significant decrease of IgG switch Dsg 1 memory B cells. Accordingly, Dsg 1 ASC were no longer detected in patients in CR at M16. Interestingly, Dsg 3-specific ThF cells were detected in patients after treatment, with an increased proportion of activated ThF2/ThF17 cell subsets. Strikingly, the frequency of autoreactive B cells was increased in CR, independently of the type of treatment. The combination of high expression of IgG switch Dsg 1 and 3 and the presence of ThF cells in CR patients indicates that RTX treatments induce the development of the memory B cells that persist in CSU patients following successful treatments.