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Transforming growth factor- β produced by keratinocytes undergoing apoptosis promotes skin fibrosis in chronic graft-versus-host disease-like reactionA Saito¹, M Fujimoto¹ and N Okiyama² *1 Dermatology, Tsukuba University, Tsukuba, Japan and 2 Department of Dermatology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan*

Scleroderma is one of the main clinical features of chronic graft-versus-host disease (cGVHD), in which donor immune cells react against host tissues after allogeneic hematopoietic stem cell transplant. It has been reported that interferon (IFN)- γ released by donor T cells promotes infiltration of donor T cells themselves into the skin, and mediates production of transforming growth factor (TGF)- β from macrophages in the pathogenesis of sclerodermatous cGVHD. To investigate the roles of host keratinocytes, which are the targets for donor T cells, in skin fibrosis of cGVHD, we established a new murine model of sclerodermatous cGVHD using transgenic mice that express membrane-bound chicken ovalbumin (OVA) under the control of a keratin 14 promoter (K14-mOVA Tg mice), which present cGVHD-like scleroderma 28 days after the transfer of OVA-specific CD8 T cells (OT-I cells). IFN γ ^{-/-}OT-I cells-transferred K14-mOVA Tg mice developed significantly milder scleroderma (clinical score, 5.9 \pm 0.64, P <0.01), which were also assessed by dermal thickness, the numbers of infiltrating α -smooth muscle actin-positive myofibroblasts and hydroxyproline contents, than wild-type OT-I cell-transferred mice (clinical score, 3.1 \pm 0.62). Moreover, mRNA expression of TGF- β were significantly lower in both total skin samples and epidermal keratinocytes from IFN γ ^{-/-}OT-I cell-transferred K14-mOVA Tg mice than in those of OT-I cell-transferred mice. Primary murine keratinocytes undergoing apoptosis produced more amount of TGF- β when stimulated by IFN- γ (179.2 \pm 12.15 pg/ml, measured by an enzyme-linked immunosorbent assay, P <0.01) as well as when treated with an apoptosis-induce agent (AT101; 107.8 \pm 12.18 pg/ml, P <0.01), compared to untreated keratinocytes (17.8 \pm 5.5 pg/ml) and borate-treated keratinocytes undergoing necrosis (0 pg/ml). Collectively, TGF- β produced by keratinocytes undergoing IFN- γ -induced apoptosis is implicated in the pathogenesis of sclerodermatous cGVHD.

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Increased frequency of CD4⁺ tissue resident memory T cells in skin lesion of lupus erythematosus and the underlying mechanismH Wu, Q Li, M Zhao and Q Lu *Dermatology, Second Xiangya Hospital, Central South University, Changsha, China*

Lupus erythematosus (LE) is a spectrum disease, from skin manifestation (discoid LE, DLE), subcutaneous LE (SACLE) to systemic involvements (SLE). The pathogenesis of LE has been intensively studied. However, the current knowledge of aberrant effector T cells cannot explain the relapse of LE, resulting in difficulties in treatment. Tissue resident memory T (TRM) cells are a type of T cells resident in tissue, differentiated from effector T cells and cannot return to the circulation. Increasing evidence has shown a critical role of TRM cells in the relapse of psoriasis and vitiligo. In our study, in the skin lesion from DLE, SACLE and SLE patients, we found a dramatically increased frequency of TRM cells in dermis, compared with healthy controls and psoriasis patients. DLE patients show the highest frequency of TRM cells (p < 0.01). When we sorted CD4⁺ or CD8⁺ TRM cells from dermis from SLE skin lesion, we found that compared with healthy controls, statistically increased CD4⁺ TRM cells were found in SLE patients (p < 0.05), rather than CD8⁺ TRM cells. When we sequenced the CD4⁺ TRM cells by single cell sequencing, we found that 46 genes were up-regulated and 60 genes were down-regulated in SLE CD4⁺ TRM cells. Among these genes, absent in melanoma 2 (AIM2) was obviously high expressed by SLE CD4⁺ TRM cells. This phenomenon is also confirmed in SLE skin lesion (p < 0.01) by multi-color IHC with PerkinElmer Vectra. In addition, AIM2 was found to be regulated by IL-21 induced TET2 enrichment on the promoter region of AIM2, and TET2 and IL-21 was also observed to be expressed wildly in lupus skin lesion. Our findings indicate that increased CD4⁺ TRM cells might contribute to the relapse of LE, providing potential biomarkers and therapeutic targets.

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Anti-thyroid peroxidase antibodies target a cytoplasmic protein in keratinocytes and may contribute to blister formation in pemphigus vulgarisK Seiffert-Sinha, S Sellitto, J Light, MM Leiker and AA Sinha *Dermatology, University at Buffalo, Buffalo, New York, United States*

In addition to having reactivity to the classical targets, desmoglein (Dsg)3 and -1, there is accumulating evidence that Pemphigus vulgaris (PV) patients harbor non-Dsg autoantibodies. The disease relevance of non-Dsg targets in PV is under investigation. We have shown that PV patients carry anti-thyroid peroxidase (TPO) antibodies at significantly higher levels than healthy controls and that anti-TPO reactivity is driven by HLA status and the absence of Dsg-reactivity. We have also shown functional effects of anti-TPO on cell adhesion and cellular processes associated with blister formation in human keratinocytes. TPO is known as an enzyme primarily involved in iodination of thyroglobulin in the thyroid gland with no known function in the skin. The exact target of anti-TPO antibodies in skin remains unclear. To clarify, we assessed TPO mRNA and protein expression by RT-PCR, Western Blot (WB) and immunofluorescence (IF) in the human keratinocyte cell line HaCaT. We show that TPO is indeed expressed at the mRNA level in HaCaT cells by PCR. At the protein level, we show definitive cytoplasmic staining with anti-TPO Abs, with no overlap with anti-Dsg3 binding or cytoskeletal components by IF. Interestingly, by Western Blot, we detect binding of anti-TPO antibody to a protein of ~75kDa size, which is not the predicted size for the canonical TPO variant (103 kDa). Variants of TPO of sizes close to 75 kDa have been described. Additionally, a number of TPO orthologs such as lacto-, myelo- and eosinophil peroxidase exhibit a high degree of sequence similarity to TPO ectodomains. Our data conclusively indicate that anti-TPO antibodies do target a keratinocyte-associated molecule, which may be a thus far unknown isoform of TPO or a structurally similar target antigen. Precise molecular characterization of this molecule will be a necessary step to understand the mechanisms by which anti-TPO autoantibodies modulate keratinocyte function in the context of PV.

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Gastrointestinal amyloidosis by long-lasting inflammatory skin diseaseK Mizutani, Y Matsushima, K Habe, K Yamanaka, K Okada and M Kondo *Dermatology, Mie University Graduate School of Medicine, Tsu, Mie, Japan*

We recently demonstrated that persistent release of IL-1ab from inflammatory skin causes weight loss, vascular sclerotic changes, and severe systemic amyloidosis in multiple organs. We addressed this question by using skin inflammatory mouse model, keratin-14 driven caspase-1 transgenic mice (KCASP1Tg). In inflammatory diseases such as rheumatoid arthritis, secondary intestinal amyloidosis occurs which causes malabsorption and bowel movement obstruction. Also in KCASP1Tg mice, long-lasting expression of IL-1 β causes gastrointestinal amyloidosis. Usually, gastrointestinal amyloidosis is irreversible in many cases and early treatment of primary disease is most important for prevention it. Here, we investigated which anti cytokine antibody is effect for prevention of gastrointestinal amyloidosis.

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Th/Tc9 and Th/Tc17 pathways are augmented in moderate-to-extensive bullous pemphigoid patients and suppressed by bertiilimumabN Kameyama¹, H He¹, H Kim¹, T Czarnowicki¹, AB Pavel¹, Y Estrada¹, J Gonzalez², AS Fiorino³, B Kolatch³, AA Sinha⁴, K Seiffert-Sinha¹, JG Krueger² and E Guttman-Yassky¹ *1 Icahn School of Medicine at Mount Sinai, Forest Hills, New York, United States, 2 Rockefeller University, New York, United States, 3 Immune Pharmaceuticals, Inc., Fort Lee, New Jersey, United States and 4 University of Buffalo, Buffalo, New York, United States*

Bullous pemphigoid (BP) is an autoimmune blistering disease with an unmet need for safe chronic therapies. BP pathogenesis and blisters involve eotaxin-1, an eosinophil chemo-attractant. Bertiilimumab, a human anti-eotaxin-1 monoclonal antibody, recently demonstrated clinical improvement in moderate-to-extensive BP patients in a phase 2a clinical trial. To evaluate cellular and cytokine polarity of BP at baseline and after bertiilimumab treatment, we analyzed peripheral blood from 8 BP patients at days 0, 42/84 of treatment, as well as 9 healthy controls, using flow cytometry. Circulating skin-homing/CLA⁺ and systemic/CLA⁺CD4⁺CD8⁺T-cell subsets were measured, as well as B-cells. We found prominent baseline CLA⁺Th/Tc9 and CLA⁺Th/Tc17 polarization in BP patients compared to controls (p <0.05), with no significant differences in Th/Tc1, Th/Tc2, and Th/Tc22 subsets. In B-cells, we found decreases in transitional (high expression of CD24/CD38) and naïve (intermediate expression of CD24/CD38) cells, and increases in IgE⁺/IgG4⁺ cells. Th9/Tc17 cells and IgE⁺/IgG4⁺B-cells correlated positively with BP disease area index/BDPAI. BP180 antibodies positively correlated with Tc2/Tc17. At day 42 of bertiilimumab treatment, we found significant decreases in CLA⁺Th9, CLA⁺Tc9, and CLA⁺Th/Tc17 frequencies (p <0.05). BDPAI improvement correlated with decreases in Tc17, as well as IgG4⁺ and IgE⁺B-cells (p <0.05). BP180/BP230 improvement also correlated with decreased Th/Tc2. The pathogenesis of BP may involve Th/Tc9 and Th/Tc17 cytokine activation, which are suppressed by eotaxin-1 blockade, suggesting its therapeutic potential for BP and beyond.

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The sand fly *Lutzomyia longipalpis* LJM17 protein induces cross-reactive antibodies against desmoglein-1 in Fogo SelvagemLA Diaz⁶, P Prisyanyh⁷, B Quaquish⁸, B Temple⁹, V Aoki⁴, G Hans-Filho⁵, E Rivitti⁴, H Friedman⁶, M Karetick⁷, S Herbert¹ and J Valenzuela⁷ *1 Dermatology, UNC, Chapel Hill, North Carolina, United States, 2 Biostatistics, UNC, Chapel Hill, North Carolina, United States, 3 Biochemistry, UNC, Chapel Hill, North Carolina, United States, 4 U Sao Paulo, Sao Paulo, Brazil, 5 U MG Sul, Campo Grande, Brazil, 6 U Brasilia, Brasilia, Brazil, 7 NIAID-NIH, Rockville, Maryland, United States and 8 Dermatology, UNC, Chapel Hill, North Carolina, United States*

Fogo Selvagem (FS) is a lethal skin disease mediated by pathogenic IgG4 autoantibodies to desmoglein-1 (Dsg1). FS is prevalent in certain regions of Brazil, where Leishmaniasis and Chagas disease are endemic. Normal individuals and leishmaniasis patients from these regions possess non-pathogenic anti-Dsg1 antibodies. They are chronically exposed to bites of the sand fly *Lutzomyia longipalpis* (LL), which elicits an IgG response to LL salivary proteins LJM17 and LJM11 and could contribute to the development of FS. We tested the antibody response to rDsg1 (IgG), and rLJM17 (IgG4) in normal settlers (n=100), FS patients (n=68) from Limao Verde (LV), an endemic focus of FS, and distinct non-endemic control populations [Brazil (n=33), USA (n=111) and Japan (n=70)]. We also studied the antibody response in mice to these antigens and assessed the protein sequence homology between LJM17 and human Dsg1. We demonstrate that healthy individuals and FS from LV had higher values of IgG4 anti-LJM17 antibodies than control groups from non-endemic areas (p <0.001, both). Levels of IgG anti-Dsg1 and IgG4 anti-LJM17 antibodies were positively correlated in normal settlers (r =0.56) and FS (r =0.38). Mice immunized with rLJM17 produce IgG1 antibodies (human IgG4 homology) that strongly cross-react with rDsg1. These cross-reactive antibodies were purified by rDsg1-Ni-agarose media and were inhibited by rDsg1 and rLJM17 in a dose-dependent manner. In contrast, rLJM11 immunized mice did not generate cross-reactive antibodies. In addition, we identified short regions of sequence homology between the ectodomain of human Dsg1 and LJM17. Inoculation of LJM17 from LL may elicit anti-Dsg1 cross-reactive IgG4 antibodies that lead to FS in a subset of genetically predisposed individuals