**Adaptive and Auto-Immunity | ABSTRACTS**

**037**

Transforming growth factor-β produced by keratinocytes undergoing apoptosis promotes skin fibrosis in chronic graft-versus-host disease-like reaction

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Keratinocytes are 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 keratinocytes in the pathogenesis of scle-rodermatous 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 scle-rodermatous cGVHD. To clarify, we assessed TGF-β production by keratinocytes undergoing IFNγ-induced apoptosis in the pathogenesis of scle-rodermatous cGVHD.

**038**

Gastrointestinal amyloidosis by long-lasting inflammatory skin disease

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We recently demonstrated that persistent release of IL-1β from inflammatory skin causes weight loss, vascular sclerotic changes, and severe systemic amyloidosis in multiple organs. IL-1β is a key inflammatory cytokine involved in the development of capase-1 transgenic mice (KACSAP1Tg). In inflammatory diseases such as rheumatoid arthritis, secondary intestinal amyloidosis occurs which causes malabsorption and bowel movement obstruction. Also in KACSAP1Tg mice, long-lasting expression of IL-1β causes gastrointestinal amyloidosis. Usually, gastrointestinal amyloidosis is irreversible in many cases and early treatment of primary disease is most important for prevention. Here, we investigated which anti cytokine antibody is effect for prevention of gastrointestinal amyloidosis.

**039**

Increased frequency of CD4+ tissue resident memory T cells in skin lesion of lupus erythematosus and the underlying mechanism

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Lupus erythematosus (LE) is a spectrum disease, from skin manifestation (discoid LE, DLE), subcutaneous LE (SLE) to systemic involvements (SLE). The pathogenesis of LE has been intensively studied. However, the current knowledge of aberrant effector T cells cannot explain the release of LE, resulting in difficult 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, SLE and LE 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). While the detection of CD4+CD8+ T 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 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 widely 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.

**040**

Th17/Tc9 and Th17/Tc17 pathways are augmented in moderate-to-extensive bullous pemphigoid patients and suppressed by bertilimumab

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Bullous pemphigoid (BP) is an autoimmune blistering disease with an unmet need for safe chronic therapies. BP pathogenesis and blisters involve eotaxin-1, an enosinophil chemo-attractant. Bertilimumab, 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 bertilimumab 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+/Th9/Tc7 and CLA+/Th17/Tc17 polarization in BP patients compared to controls (p < 0.05), with no significant differences in Th1 cells, Th17 cells, and Th1/Th2 cells subsets. In B-cells, we found decreases in transitional (high expression of CD4/CD38) and naive (intermediate expression of CD4/CD38) cells, and increases in IgE+/IgG4+ cells. Th9/Tc7 cells and IgE-/IgG4+B-cells correlated positively with BP disease area index/BPDAI. BP180 antibodies positively correlated with decreases in CLA+/Th9, CLA+/Th7, and CLA+/Th17/Tc7 frequencies (p < 0.05). BPDAI improvement correlated with decreases in Th17, as well as IgG4- and IgE-B-cells (p < 0.05). BP180/BPDAI improvement also correlated with decreased Th17/Tc2. The pathogenesis of BP may involve Th1/Th17/Tc7 cytokine activation, which are suppressed by eotaxin-1 blockade, suggesting its therapeutic potential for BP and beyond.

**041**

Anti-thyroid peroxidase antibodies target a cytoplasmic protein in keratinocytes and may contribute to blister formation in pemphigus vulgaris

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In addition to having reactivity to the classical targets, desmoglein (Dsg)1 and -3, 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 line HaCat. We show that TPO is indeed expressed at a low 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 antibodies to an antibody size, which is not consistent with a thyroid-specific protein (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 ectodomain. Our data conclusively indicate that anti-TPO antibodies target a cytoplasmic protein in the context of PV.

**042**

The sand fly Lutzomyia longipalpis LJM17 protein induces cross-reactive antibodies against desmoglein-1 in Fogo Selvagem

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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 Lut-zomyia longipalpis (LL), which elicits an IgG response to FS,(LL) and FS(LL) and LJM11 immunized mice did not generate cross-reactive IgG4 responses. In contrast, LJM17 immunized mice generated IgG4 antibodies that cross-reacted with Dsg1. Indeed, we have shown that recombinant LJM17 (rLJM17) binds to Dsg1 with high affinity and induces cross-reactive IgG4 antibodies that lead to FS in a subset of genetically predisposed individuals.