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

K. Saito1, T. Okajima1, T. Tani1, M. Mie, T. Isoda1, M. Suzuki1, S. Noguchi1, N. Sato1, A. Yano2, K. Goh2, S. Ohashi2, K. Inoue, S. Furukawa2, K. Nishino2, C. Kudo1, T. Tsukuba1 and T. Tsukuba1

Sclerodermatous cGVHD, 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-β (TGF-β) from keratinocytes 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 expressing transmembrane-biotin (OVAb) under the control of a keratin 14 promoter (K14-OMoVA Tg mice), which present cGVHD-like scleroderma 28 days after the transfer of OVA-specific CDB T cells (OT-I cells). IFNγ+ OT-I cells-transferred K14-OMoVA Tg mice developed significantly milder scleroderma-like fibrosis (p<0.01), which was 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±0.62). Moreover, mRNA expression of TGF-β were significantly lower in both total skin samples and epidermal keratinocytes from IFNγ+ OT-I cells-transferred K14-OMoVA 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 (17.9±2.12 pg/ml, measured by an enzyme-linked immunosorbent assay, P<0.01) as well as when treated with an apoptosis-inducer agent (AT101; 107.8±0.05 pg/ml, P<0.01), compared to untreated keratinocytes (17.8±5.5 pg/ml) and bortate-treated keratinocytes undergoing necrosis (0 pg/ml). Collectively, TGF-β found in SLE patients (AT101; 107.8±0.05 pg/ml, 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±0.62). Moreover, mRNA expression of TGF-β were significantly lower in both total skin samples and epidermal keratinocytes from IFNγ+ OT-I cells-transferred K14-OMoVA 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 (17.9±2.12 pg/ml, measured by an enzyme-linked immunosorbent assay, P<0.01) as well as when treated with an apoptosis-inducer agent (AT101; 107.8±0.05 pg/ml, P<0.01), compared to untreated keratinocytes (17.8±5.5 pg/ml) and bortate-treated keratinocytes undergoing necrosis (0 pg/ml). Collectively, TGF-β produced by keratinocytes undergoing IFNγ-induced apoptosis is implicated in the pathogenesis of sclerodermatous cGVHD.

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

H. 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 (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 escape 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, 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). When we detected CD8+ or CD4+ 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 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.

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

K. Seiffert-Sinha, S. Jellott, M. Leiker and A. Sinha Dermatology, University of Buffalo, Buffalo, New York, United States

In addition to having reactivity to the classical targets, desmoglein (Dsg) 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-reactive antibodies. In addition, we identified short regions of sequence homology between the TPO ectodomain of human Dsg1 and LJM17. Inoculation of LJM17 from R. Lutzomyia longipalpis (LL) could contribute to the development of FS. We tested the antibody response to rDsg1 (IgG), and rLJM17 (IgG) in normal settlers (n=111) and FS patients (n=100), FS patients (n=68) from L. longiseta (LL), 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 LL and human Dsg1. We demonstrate that healthy individuals and FS from LL had higher values of IgG anti-LJM17 antibodies than control groups from non-endemic areas (p<0.001, both). Levels of IgG anti-Dsg1 and IgG 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 homolog] that strongly cross-react with rDsg1. These cross-reactive antibodies were purified by rDsg1Ni-agarose media and were inhibited by rDsg1 and rLJM17 in a dose-dependent manner. In contrast, rLJM17 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 IgG antibodies that lead to FS in a genetically predisposed individuals.