001 Maintenance of CD4+ tissue-resident memory T cells via perivascular clusters with CD301b+ dermal dendritic cells in a mouse model of allergic dermatitis

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Tissue-resident memory T cells (T(RM)) play a crucial role in local immunity by inducing rapid immune responses without the exposure of the antigen. However, how CD4+ T(RM) cells retain in the skin after allergic inflammation remains largely unknown. To clarify the mechanism, we used a delayed-type hypersensitivity model, which is mediated by CD4+ T cells. T cell receptor (TCR)-β deficient mice were transferred with CD4+ T cells from GFP-expressing, ovalbumin (OVA)-specific TCR-transgenic (OT-II) mice and sensitized with OVA emulsion, followed by initial challenge with OVA in ear skin (day 0). On day 35, in spite of the resolution of ear swelling, CD4+ T cells remained in the dermis and exhibited CD4+CD62L−CD103hi cells. Their residence was associated with fibrosis and parabiosis and photo-convertible protein (Ki67)-expressing OT-II T cells. In addition, two-photon microscopy revealed that CD4+ T cells were retained in perivascular clusters on day 35. Immunohistochemical analysis revealed that CD301b+ conventional dendritic cell (DC) subset 2 (CDD2) expressed CD103 on cell surface and were associated with CD301b+ cells in Agg2-diphtheria toxin receptor (DT) mice, which express the DTR under the regulation of the gene encoding CD301b, and found that the number of CD4+ T(RM) cells and their clusters were reduced after the depletion of CD301b+ cells. Taken together, these results suggest that CD301b+CD2 cells are critical in the tissue residency of CD4+ T(RM) cells after the resolution of allergic inflammation. This mechanism provides a potential new strategy for preventing the recurrence of CD4+ T cell-mediated chronic inflammatory skin diseases.

002 Skin-resident memory T cells are poised for systemic Th2/Th17-driven inflammation and may re-seed at distant sites during graft-versus-host disease

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We previously showed that bridomas of spontaneously activated B cells were screened for antibodies against other possibly autoreactive epitopes. Here, we further mapped the antigen. Western blot analysis and IIF staining using skin from patients with GVHD revealed the presence of DSG3 and p53, which are key components of the pathogenesis of PV. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of p53. Second, we showed that DSG3 down-regulation activates the TSH pluripotent phenotype in skin cells. Thus, we identified a potential new target for the prevention of GVHD.

003 Anti-fractalkine monoclonal antibody therapy ameliorates murine sclerodermatous chronic graft-versus-host disease

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Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis and vascular injury of skin and internal organs. Fractalkine (CX3CL1) is a chemokine that is expressed on vascular endothelial cells and functions as an adhesion molecule for CX3CR1-positive leukocytes. Additionally, soluble fractalkine is involved in the migration of CX3CR1-expressing leukocytes into the lesional tissue. We previously reported that the expression of fractalkine and its receptor CX3CR1 was both augmented in SSc patients and anti-mouse fractalkine monoclonal antibody (mAb) suppressed inflammation, fibrosis, and vascular injury of the skin in bleomycin- or TGF-β-induced SSc murine models. However, these models do not develop apparent fibrosis and inflammation in internal organs. In the current study, we investigated the utility of anti-mouse fractalkine therapy in a murine sclerodermatous chronic graft-versus-host disease (Scl-cGVHD) model. Allogeneic bone marrow transplantation into sublethally irradiated BALB/c mice reproducibly induced organ fibrosis and autoimmune phenotypes resembling human Scl-cGVHD or SSc. An intraperitoneal administration of anti-fractalkine mAb increased survival rate in a dose-dependent manner. The mAb therapy significantly suppressed the fibrosis of the skin and lungs. Moreover, the mAb dose-dependently attenuated the local infiltration of T lymphocytes in the skin and lungs macrophages in the lungs. Furthermore, the mAb inhibited the expression of proinflammatory cytokines such as IL-6, TNF-α, and prostatic cytokine IL-4 in the skin and the TNF-α expression in the lungs. Anti-fractalkine mAb treatment did not show any apparent adverse events. These data together support the possible role of fractalkine in the development of Scl-cGVHD and SSc.

004 Up-regulation of ST18 drives pemphigus vulgaris pathogenesis: a perpetuum mobile model

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Pemphigus vulgaris (PV) is a life-threatening autoimmune mucocutaneous blistering disease. Autoimmune blistering is associated with increased p53 expression and activity. Based on these data, using a combination of reporter assays, Western blotting, confocal immunofluorescence microscopy, we investigated the possibility that ST18, DSG3 and p53 may be jointly involved on the pathogenesis of PV. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of p53. Second, we showed that DSG3 down-regulation activates the ST18 pluripotent phenotype, which provides evidence of a potential new target for the prevention of GVHD.

005 PPAR-γ promoter-mutations promote proliferation of pathogenic Th2 cells through regulation of IL-2 signaling

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We previously showed that a genetic variant within the ST18 promoter promotes ST18 up-regulation in the setting of autoimmune blistering diseases. First, we demonstrated that antibody-mediated DSG3 down-regulation results in increased p53 expression and activity. Based on these data, using a combination of reporter assays, Western blotting, confocal immunofluorescence microscopy, we investigated the possibility that ST18, DSG3 and p53 may be jointly involved on the pathogenesis of PV. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of p53. Second, we showed that DSG3 down-regulation activates the ST18 pluripotent phenotype, which provides evidence of a potential new target for the prevention of GVHD.

006 Pathogenic autoantibody derived from Treg-deficient scurfy mice targets type VII collagen and induces Epidermolysis bullosa acquiesita-like blistering disease

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Scurfy mice have a missense mutation in the transcription factor Foxp3 which leads to dysfunctional Tregs. Previously, we have shown that scurfy mice develop high titers of autoantibodies with reactivity to structural proteins in the skin. Furthermore, the development of a pathogenic autoantibody that targets BP230 and induces a bullous pemphigoid-like phenotype indicates the progression of autoimmune blistering diseases (AIBD) in the absence of functional Tregs. The aim of this study was to investigate the role of Tregs in the pathogenesis of AIBD. We previously showed that a genetic variant within the ST18 promoter promotes ST18 up-regulation in the setting of autoimmune blistering diseases. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of p53. Second, we showed that DSG3 down-regulation activates the ST18 pluripotent phenotype, which provides evidence of a potential new target for the prevention of GVHD.

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