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 (T RM) cells play a crucial role in local immunity by inducing rapid immune responses upon exposure of the antigen. However, how CD4+ T RM cells retained 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 3, in spite of the resolution of ear swelling, CD4+ T cells remained in the dermis and exhibited CD4+CD8- signature of CD8α+ T cell memory. Their redistribution, survival, and proliferation in parallel to photo-convertible protein KitGK-GR 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 (cDC) subset 2 (cDC2) cells colocalized with CD4+ T cells in the clusters. We selectively depleted CD301b+ cells and their clusters were reduced after the depletion of CD301b+ cells. Taken together, these results suggest that CD301b+ cDC2 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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Skin-resident memory T (TRM) cells are thought to be non-circulating cells providing rapid recall responses against outside pathogens. Recent observations in humanized mouse models indicate that a subset of tissue-resident memory T cells (Treg) may exit the skin and form a discrete circulating T cell population in the blood. To investigate whether re-circulating TRM population in humans, we characterized circulating T cells with a skin TRM phenotype in the blood of patients after allogeneic hematopoietic stem cell transplantation (HSCT). We found a small and stable population of CD4+CD103+CLAla+ T cells in the blood of all patients analyzed and verified their tissue origin by genetic analysis in sex-mismatched HSTC recipients. Transcriptional analysis on single-cell level revealed their striking resemblance to skin TRM. Blood from patients with GVHD contained elevated numbers of host-derived CD4+CD103+CLAla+ T cells producing pro-inflammatory Th2/Th17 cytokines, which highlights the potential of skin TRM to contribute to inflammation on a systemic level. Importantly, gastrointestinal GVHD lesions contained CD4+CD103+ TRM expressing the cutaneous leucocyte antigen CLA, indicating potential re-seeding of skin TRM in the gut. Collectively, our data offers first proof of a distinct Trm-like circulating T cell type, which mirrors cutaneous inflammation and may disseminate disease via the blood circulation.

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

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Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis and vascular injury of the 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 a sublethally irradiated BALB/c mice reproduced 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 and macrophages in the lungs. Furthermore, the mAb inhibited the expression of proinflammatory cytokines such as IL-6, TNF-α, and profibrotic 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 with our previous findings in other mouse models indicate that the systemic administration of anti-fractalkine mAb can be an attractive therapeutic approach for human 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. We previously showed that a genetic variant within the ST18 promoter promotes ST18 up-regulation in a ps3/p63-dependent manner and is associated with a 6-fold increased risk to develop PV. ST18 was also found to be overexpressed in the skin of patients. In addition, it has been shown that desmoglein 3 (DSG3) down-regulation is associated with increased ps3 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 p63 may be jointly involved on the pathogenesis of PV. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of ps3. Second, we showed that DSG3 down-regulation activates the ST18 promoter activity. Third, ps3 silencing abolished the DSG3-mediated activation of the ST18 promoter activity. Finally, we demonstrated that ST18 overexpression in keratinocytes significantly augments antibody-mediated DSG3 down-regulation in keratinocytes. Taken collectively, these data indicate that ST18 up-regulation triggers a pathophysiological self-amplifying cycle involving DSG3 and ps3 dysregulation, which may underlie the genetic association of ST18 variants with PV. Supporting the clinical relevance of these findings, a genetic variant causing increased ST18 promoter activity was found to be associated (p = 0.003) with more severe phenotype in a cohort of 100 PV patients.

005 Pathogenic autoimmune dendritic cell derived from Treg-deficient scurfy mice targets Type VII Collagen and induces Epidermolysis bullosa acquistica-like blistering disease

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Missing regulatory T cell (Treg) control contributes to the development of different autoimmune diseases. Scurfy mice have a missense mutation in the transcription factor Foxp3 which leads to dysregulatory Treg. Previously, we have shown that scurfy mice develop high titers of autoantibodies with reactivity against structural proteins in the skin. Furthermore, the development of a pathogenic autoimmune dendritic cell that targets BP210 and induces a bullous nephropathic phenotype indicates the progression of autoimmune blistering diseases (AIBD) in the absence of functional Treg. Here, using a scurfy mouse model of autoimmune disease in scurfy mice, bybredomas of spontaneously activated B cells were screened for antibodies against other potential pathogenic target antigens. We found a murine IgGc, autoantibody (H510) which is pathogenic in vivo as it induces subepidermal blisters in neonatal mice and binds to the blister floor on murine salt-split skin in indirect immunofluorescence (IF). After we excluded that H510 binds to laminin-332, we identified type VII collagen (Col7) as a potential auto-antigen. Western blot analysis and IF staining using skin from Col7-knockout mice revealed the presence of autoreactive antibodies against collagen VII. We further mapped the pathogenic epitope to the murine von-Willebrand-factor-A-like domain 2 of Col7. In summary, we here present a recently identified, pathogenic autoimmune dendritic cell spontaneously developed in the absence of functional Treg cells with reactivity against Col7 as a useful model for the induction of Epidermolysis bullosa acquistica in mice.

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