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

R Kakehashi, G Egawa, S Nakamizo and K Kabashima Dermatology, Kyoto University, Kyoto, Japan

Tissue-resident memory T (T RM) cells play a crucial role in local immunity by inducing rapid immune responses upon exposure to 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 35, in spite of the resolution of ear swelling, CD4+ T cells remained in the dermis and exhibited CD44+CD69+ TRM cell phenotype. Their residency was correlated with the fibrosis and para-crine and photo-convertible protein (Kigk(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 (DC) subset 2 (cDC2) cells co-localized with CD4+ T 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+ TRM 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

K Keusgen, D Kaminski, P Schirmacher and HK Hoffmann

Skin-resident memory T (T RM) 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 (Trm) may exit the skin and form a discrete circulating T cell population in the blood. To test if of 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+CLA+ T cells in the blood of all patients analyzed and verified their tissue origin using genetics and sex-matched control. These CD103+ cells in Mgl2-deferlithia toxin receptor (DTR) mice, which express the DTR under the control of the gene encoding CD103, and found that the number of CD4+ T RM cells and their clusters were reduced after the depletion of CD301b+ T 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.

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

A Utsunomiya1, V Huy Luong1, T Chino1, N Oyama1, T Matsushita2, N Ishii3, H Ogasawara3, T Morimoto1, T Sato1, T Kodama2, H Iwamoto1, K Sugiyama1, N Yamaguchi1, T Fujita1, T Kato1, T Saito1, T Fuchigami1, W Ishii1, K Takenouchi1 and Y Honjo1

Sclerodermatous chronic graft-versus-host disease (scl-cGVHD) is a severe GVHD with long-lasting cutaneous inflammation and may disseminate disease via the blood circulation. We previously showed that a genetic variant within the STAT3 promoter promotes STAT3 up-regulation in a p53/p63-dependent manner and is associated with a 6-fold increased risk to develop GVHD. STAT3 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 p53 expression and activity. Based on these data, using a combination of reporter assays, Western blotting, confocal immunofluorescence microscopy, we investigated the possibility that STAT3, DSG3 and p53 may be jointly involved on the pathogenesis of scl-cGVHD. First, we demonstrated that antibody-mediated DSG3 down-regulation results in enhanced expression of p53. Second, we showed that DSG3 down-regulation activates the STAT3 promoter activity. Third, p53 silenced abolished the DSG3-mediated activation of the STAT3 promoter activity. Finally, we demonstrated that STAT3 overexpression in keratinocytes significantly augments antibody-mediated DSG3 down-regulation in keratinocytes. Taken collectively, these data indicate that STAT3 up-regulation triggers a pathophysiological self-amplifying cycle involving DSG3 and p53 dysregulation, which may underlie the genetic association of STAT3 variants with GVHD. Supporting the clinical relevance of these findings, a genetic variant causing increased STAT3 promoter activity was found to be associated (p<0.003) with a more severe phenotype in a cohort of 100 patients.

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

S Assaf1, J Mohammadi2, L Malik1, K Malovitch3, O Sarig1, D Vodoi1 and E Sprecher1

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 p53/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 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 STAT3 promoter activity. Third, p53 silenced abolished the DSG3-mediated activation of the STAT3 promoter activity. Finally, we demonstrated that STAT3 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 p53 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 a more severe phenotype in a cohort of 100 patients.

005 PPAR-gamma promotes proliferation of pathogenic Th2 cells through regulation of IL-2 signaling

F Luther1, NL Bertchi1, O Steck1, C Razini1, I Keller1 and C Schlaphack1

1 Dermatology, Innsbruck, 2 University Hospital, Berne, Switzerland and 2 Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland

Recently, a subset of allergen-specific Th2 cells has been identified and termed “atopogenic” Th2 (pTh2) cells, based on their crucial role in mediating type-2-mediated immunopathology. pTh2 cells express high levels of the ligand-activated transcription factor peroxisome proliferator-activated receptor gamma (PPAR-g). The functional role of PPAR-g for pTh2 cells, however, remains incompletely understood. Here, we analyzed the effect of PPAR-g inhibition on basic T cell functions such as IL-2- or T cell receptor (TCR)-induced proliferation in pTh2 cells isolated from peripheral blood. Strikingly, PPAR-g inhibition strongly reduced IL-2-induced proliferation, but not TCR-induced proliferation, suggesting specific control of cytokine signaling events by PPAR-g. To investigate the underlying mechanism, we performed transcriptomic analysis of T cell clones treated with a chemical inhibitor (GW9662) of PPAR-g. Pathway analysis revealed that the IL-2 signaling pathway is affected by PPAR-g inhibition, in line with our observation from the proliferation data. To assess the impact of PPAR-g inhibition on IL-2 signaling, we systematically measured its effect on phosphorylation of signal transducer and activator of transcription (STAT) molecules. Cells treated with GW9662 showed a significantly reduced phosphorylation of STAT3 and STAT5, while phosphorylation of STAT6 remained unaffected. Together, our findings suggest that PPAR-g is a positive regulator of the IL-2 signaling pathway in pTh2 cells. Since IL-2 is crucial for T cell proliferation and Th2 cell development, these findings might provide an explanation for pTh2 overproliferative Th cells under conditions of limited IL-2 availability in the tissue. These findings further highlight the potential of PPAR-g as a therapeutic target in type 2 immunopathology.

006 Pathogenic autoantibody derived from Treg-deficient scurfy mice targets Type VII Collagen and induces Epidermolysis bullosa acquisita-like blistering disease

E Vicari1, S Haebel2, V Bokd1, T Ramcke2, A Voehr2, SG Goel2, S Hwatoa2, R Ludwig1, EM Schmidt1, A Enk3 and E Hadaczek1

1 Dermatology, Heidelberg University Hospital, Heidelberg, Germany, 2 Dermatology, University medical center Schleswig- Holstein, 3 Institute of Experimental Dermatology of the University of Luebeck, 4 Dermatology, Faculty of Medicine and Graduate School of Medicine Hokkaido University, Sapporo, Japan and 5 Dermatology, Essen University Hospital, Essen, Germany

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 dysfunctional Tregs. Previously, we have shown that scurfy mice develop high titers of auto-reactive antibodies with reactivity to structural proteins in the skin. Furthermore, the development of a pathogenic autoantibody that targets BP210 and induces a bullous pemphigoid-like phenotype indicates the progression of autoimmune blistering diseases (AIBD) in the absence of Foxp3. We therefore investigated the role of autoantibodies in the development of scurfy mice, by intravenously activated B cells were screened for antibodies against other potential pathogenic target antigens. We found a murine lgG, autoantigen (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 IIF staining using skin from Col7 knockout mice revealed that the antibody reactivity against Col7 was specific to null mice. We further mapped the pathogenic epitope to the murine Col7/Col7-containing A-domain. Taken together, these findings may provide insight into the pathogenesis of AIBD and give clues for therapeutic interventions.