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Epicutaneous sensitization with protein allergens differentiates naïve T cells into not only Th2 but also Th17 cells, which may involve in the delayed reactions in protein contact dermatitis

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Protein contact dermatitis (PCD) is caused by proteins. The patients may show positive immediate or delayed reactions in skin prick, scratch or patch test, or the skin tests may remain negative. Specific IgE is obviously crucial in most reactions, however, the exact mechanisms remains unclear. To address these issues, we investigated mice sensitized by papain enzyme as a protein allergen. Mice were sensitized by 24hrs exposure to papain twice a week for one month. The draining lymph node cells were then isolated and re-stimulated by papain. ELISA and FACS analyses of the cytokines synthesized in lymph node cells revealed that CD4+ IL4+ T (Th2) and CD4+ IL17+ T (Th17) cells were differentiated in the draining lymph nodes of the mice sensitized by papain. However, CD4+ IFN-gamma+ T cells (Th1) were not detected. The sensitized mice were elicited by applying papain onto the ear to address the role of Th2 and Th17 cells differentiated in the papain-sensitized mice, and the allergic reactions in the ear were examined. Real-time PCR analyses revealed that IL-17 mRNA of Th17 marker was up-regulated in a time-dependent manner; however, IL-4 mRNA of Th2 maker was not detected at any time points. These phenomena were confirmed by immunofluorescent microscopy using a specific antibody for IL-17, suggesting that Th17, but not Th2, migrated into the ear after the elicitation. Additionally, the papain-sensitized mice possessed papain-specific IgE, and mast cell degranulation was observed after the elicitation. These findings reveal for the first time that the sensitization of papain enzyme differentiates naïve T cells into not only Th2 but also Th17 cells as effector T cells contributing to the allergic reactions. The generated Th2 produces IL-4 and is involved in IgE syntheses in the draining lymph nodes. Conversely, the generated Th17 cells migrate to the skin and produce IL-17 after elicitation, which may involve in the delayed reactions of PCD.

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Interleukin 31 production is associated with Histamine H4 receptor of CLA$^+$ CD45RO$^+$ T cell

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Interleukin-31 (IL-31) is a recently-discovered Th2-cell-derived cytokine which can induce severe pruritus and skin lesions such as atopic dermatitis (AD). IL-31 mRNA and protein expressions are largely restricted to CD4$^+$ T cells, particularly CD45RO$^+$ (memory) CLA$^+$ T cells. However, the mechanism of IL-31 production in these cells is not fully recognized yet. Histamine-4-receptor (H4R) is a recently described type of histamine receptor upregulated under Th2 conditions, and HR4 stimulation leads to the induction of IL-31 *in vitro*. However, the mechanism and association between IL-31 and H4R in human has not been reported. The purpose of the present study is to evaluate the hypothesis that H4R$^+$ CLA$^+$ CD45RO$^+$ T cells produce IL-31 predominantly. To investigate that H4R$^+$ cells produce IL-31, immunofluorescence studies were performed on punch biopsy specimens of 5 AD, 3 prurigo nodularis and 3 healthy controls. In addition, blood samples were collected from 12 AD patients and healthy controls. The H4R$^+$ CLA$^+$ CD45RO$^+$ T cells and the other cells were sorted by fluorescence-activated cell sorting (FACS). These sorted cells were stimulated with 4-methylhistamine and the level of IL-31 in the culture supernatant were measured by enzyme-linked immunosorbent assay. Under high-power field, the IL-31$^+$ H4R$^+$ colocalized cell counts in biopsy specimens were 12.9 ± 9.2, 6.7 ± 6.0 and 0.0 ± 0.0 in AD, prurigo nodularis, and control samples, respectively ($p<0.01$). The result of FACS showed that the proportion of H4R$^+$ CLA$^+$ CD45RO$^+$ T cells were 31.5 ± 9.6% and 23.1 ± 5.2% in AD and healthy control, respectively. In AD patients, these cells showed 2.3-fold higher production of IL-31 compared to healthy control ($p<0.05$). According to our study, H4R$^+$ CLA$^+$ CD45RO$^+$ T cells are a major source of IL-31, and this subset may be targets for the treatment of IL-31 induced pruritus.

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Inhibition of phosphodiesterase-4 significantly decreases oral mucosa lesions in experimental anti-laminin 332 mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is characterized by autoantibodies against the dermal-epidermal-junction and a mucosal disease predominating over skin involvement. As the treatment of MMP patients still relies on high-dose corticosteroids, there is an unmet need for new and more specific therapies. Here, we made use of a recently established experimental model that recapitulated major clinical and immunopathological characteristics of human MMP by the injection of IgG against the murine alpha3 chain of laminin 332 (Lam332) into adult mice. In a prophylactic approach, the specific PDE4 inhibitor roflumilast (ROF) led in 2 independent, blinded experiments, to the reduction of oral lesions compared to vehicle-treated mice as quantified by endoscopy ($p=0.029$). In contrast, an increase in skin lesions was observed in ROF-treated mice ($p<0.0001$). In a quasi-therapeutic approach, i.e. when ROF/vehicle was not used until mice had developed first skin lesions, ROF-treated mice showed significantly less oral lesions compared to vehicle-treated mice, while skin lesions did not differ. To investigate the discrepant effect of ROF on oral and skin lesions, a transcriptome analysis of both tissues in ROF- and vehicle-treated anti-Lam332 MMP mice as well as mice injected with normal rabbit IgG was performed. An up-regulation of IL-6 and an impact of CXCL2 were found by Gene Set Enrichment and STRING analysis, respectively, in both the skin and buccal mucosa of vehicle-treated mice. The subsequent incubation of murine keratinocytes with anti-mLam332 IgG resulted in a dose-dependent release of IL-6 and CXCL2, which was inhibited by the addition of ROF. Our data propose IL-6 and CXCL2 as relevant pathogenic factors in MMP and suggest PDE4 inhibition as potential novel treatment options for MMP patients with severe oral lesions.

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Skin tissue resident memory T cells in HIV infection

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There is still no cure for HIV, as the virus can survive in quiescent CD4+ subsets and rebound as soon as combined antiretroviral treatment (cART) is interrupted. Current cART is so effective in stopping viral replication and allowing immune reconstitution of the CD4 T cell pool, that people living with HIV (PLWH) have a life expectancy similar to the healthy population. However, new challenges in HIV management are emerging, in particular the increased risk for developing non-HIV-related cancers, like the human papilloma virus (HPV) induced anal cancer. Tissue resident memory T cells (T_{RM}) are a critical component of our immune defence and play accumulating roles in tissue homeostasis and cancer surveillance. We therefore sought to investigate skin T_{RM} in PLWH. We collected skin biopsy and peripheral blood mononuclear cells (PBMCs) from individuals during the acute and chronic phase of HIV infection for the characterization of skin immune cells by Tissue-FACS software, flow cytometry, cell sorting, DNA and RNA analysis. Our preliminary results show that the CD4 T_{RM} numbers are strongly reduced in the skin of PLWH, despite of immune-reconstitution of the blood CD4+T cell pool upon begin of cART. Low skin CD4+ TRM numbers do not show correlation with the circulatory CD4+T cells, but rather with the nadir, i.e. the lowest blood CD4+T cell number the history of the patients. These preliminary data suggest that the skin CD4+ T_{RM} are targeted by HIV and never reconstituted, despite initiation of cART and immune reconstitution of the recirculating CD4+ cell pool. In further analysis, we will follow the skin CD4+ T_{RM} cell numbers at diagnosis and 1 year after therapy begin. We will also analyse the skin CD4 T_{RM} cells in the mucosal tissue of PLWH which underwent biopsies for HPV dysplasia screening. Our study will shed light on the mechanism of skin and mucosa immune surveillance in chronic HIV infection and might help identifying the cause of increased HPV related dysplasia in this risk population.

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Type I immunity induces a senescence-like growth arrest in malaria parasites

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Type I immunity kills and contains plasmodium species in mammalians. However, malaria tends to recur, showing that the natural type I immune response strongly reduces the pathogens but fails to eradicate them. Analyzing the effect of type I immunity on plasmodium-infected hepatocytes, we confirmed that indeed IFN (interferon)-γ strongly reduces parasite number. Surprisingly, we also found that IFN-γ induces in addition a senescence-like growth arrest in the remaining plasmodium parasites. One day of treatment with IFN-γ caused a stable growth-arrest over 14 days in surviving *Plasmodium (P.) falciparum* liver stages. IFN-γ induced small non-proliferating plasmodium forms in human, monkey and mouse hepatocytes. Atovaquone treatment killed the large majority of the remaining parasites confirming that these small forms are alive. In vitro data showed that mammalian p21 can interact with parasite cyclin-dependent kinases (CDK) PFMRK, PFPK5 and PFPK6. Inhibition of these CDKs with Artemisinin severely impairs the growth of *P.falciparum*, without killing the parasites. Therefore, we asked whether IFN-γ and plasmodium parasites induce p21 in mammalian hepatocytes and whether mammalian p21 can arrest *P.falciparum* in vivo. Liver-infection with plasmodium parasites leads to an interferon-response and induced p21 but not p16 protein levels inside primary hepatocytes. The p21 protein was further enhanced by treatment with IFN-γ and tumor necrosis factor. Most importantly, p21$^{0/0}$ hepatocytes had a major defect in containing malaria parasites in vitro. In consequence, infection of p21$^{0/0}$ mice had a major defect in controlling the onset and the extent parasitemia. Hence, type I immunity did not only kill malaria parasites but induced a p21-dependent senescence-like growth arrest in the remaining plasmodium stages. This IFN-γ induced senescence-like growth arrest significantly contributed to the containment of the disease.

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IgE in skin and serum of nonbullous and bullous pemphigoid patients

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Nonbullous pemphigoid (NBP) is a pemphigoid variant, in which for unknown reason patients do not develop blisters. In bullous pemphigoid (BP) accumulating evidence suggests a role for IgE in disease pathogenesis, and possibly in blister formation. This study assessed the presence of IgE in serum and skin of BP and NBP, using enzyme linked immunosorbent assay (ELISA) and immunofluorescence techniques. We included 67 NBP and 50 BP serum samples, and control sera of 25 pemphigus patients, and 25 elderly patients with pruritus. Total IgE was elevated in 60% and 63% of BP and NBP, and in 20% and 60% of pemphigus and elderly controls. IgE ELISAs were more frequently positive in BP than in NBP (NC16A 18% vs. 9%, p=0.139; BP230 34% vs. 22%, p=0.149). IgE optical density values were significantly higher in BP compared with NBP (p=0.000). Surprisingly, elderly controls had IgE antibodies to NC16A and BP230 in 8% and 20%, while all pemphigus controls were negative. Increased total IgE correlated with positive anti-NC16A IgE ELISA in BP (p=0.007), and positive anti-BP230 IgE ELISA in NBP (p=0.000). In elderly controls specific and total IgE did not correlate. Skin biopsies of 14 BP and 14 NBP patients with highest anti-NC16A IgE ELISA titers were stained for IgE for direct immunofluorescence microscopy. Four biopsies (14%; 2 NBP, 2 BP) showed linear IgE along the basement membrane zone, while IgE was mostly bound to the surface of dermal cells (71% NBP, 86% BP). In conclusion, IgE was present in serum and skin of both NBP and BP patients, suggesting a supportive role of IgE instead of being a key mediator of blister formation in pemphigoid.