ABSTRACT | Adaptive and Auto-Immunity

019 Functional changes in Langerhans cells (LCs) may partially explain alterations in skin immunity with aging
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Functional changes in LCs that occur in older animals may explain some of these alterations in skin immunity observed with aging. While epidermal LCs were originally seen as potent APCs for initiation of antigen (Ag)-specific immune responses, recent data suggest they may downregulate immune responses. In some situations, such as in the presence of a danger signal, they can present Ag for effector immune responses. Altered function of aged LCs has been reported. We have now compared cytokine profiles of T cells responding to Ag presentation by LCs from mice 2-3 months old versus presentation by LCs from mice >12 months old. LCs were isolated from BALB/c epidermis with magnetic antibody techniques and co-cultured with splenic CD4+ T cells isolated from 12-16 week-old DO11.10 mice (transgenic mice with T cells responsive to a fragment of chicken ovalbumin, 10 μM cOVA323-339) and 10 μM cOVA323-339. Supernatants were collected at 48 hrs and IL-6, IL-17, IL-22, and IFNγ concentrations assayed by ELISA. IL-6, IL-2A and IL-22 contents were significantly lower in supernatants containing mature LCs compared to those with young LCs (p = 0.002 for IL-6, p < 0.001 for IL-17A and p < 0.001 for IL-22). Trends toward a lower concentration of IL-4 and IFNγ were seen in supernatants from cultures containing mature LCs that did not reach statistical significance. IL-9 production was similar in the 2 groups. These data support the concept that LCs from older mice are less efficient at presenting Ag for elicitation of IL-6, IL-17A and IL-22 responses. These results may suggest a progressive inhibition of inflammatory responses with age and be consistent with disorders involving age-related disorders in skin immunity observed with aging. Indeed, there are reports that the prevalence of human psoriasis advances with increased age.

021 IL-17A and not IL-17E is required for IL-17C-mediated psoriasisiform inflammation
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Transgenic mice expressing keratinocyte-IL-17C (IL-17C+) develop a psoriasisiform skin phenotype. To explore the mechanisms underlying IL-17C-mediated inflammation, we crossed mice with IL-17A-deficient (IL-17A-/-) mice and skin examined. IL-17C+/IL-17E+KO mice had similar acanthosis (39±3 vs. 45±3μm) and CD4+ (14±1 vs. 14±2) and CD8+ (2±1 vs. 3±1) T cell numbers as IL-17C+ mice (n=8/gp; P=0.1). Moreover, acanthosis and T cells did not change in IL-17C+ mice transplanted with bone marrow from CD4+ IL-22+ IL-17E+ vs. control mice (n=6-9). These results suggest that IL-17E signaling is IL-17E independent. Next, IL-17C+IL-17E+ mice were treated with anti-IL-17A antibody, but not with IL-17E, which improved acanthosis (14±1 vs. 6±3μm; n=8/gp; P=0.0006) and skin CD4+ and T cells (8±1 vs. 19±2; P=0.001) compared to IL-17C+ mice. Interestingly, IL-17A modulation (+ or -) in IL-17C+ mice using anti-IL-17A antibodies, transplanting IL-17AKO bone marrow, IL-17E-/- mice, or introducing IL-17E intraduallarily, each had no effect on IL-17C+ skin inflammation. Together, our results demonstrate that IL-17C is critical for sustaining skin inflammation in an IL-17E independent, IL-17RA dependent manner and that a tiered balance between IL-17C- and IL-17E during skin inflammation is critical for dictating levels of inflammation.

022 Enhancing the vaccinal effect of anti-tumor antibodies in melanoma
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Melanoma is the most aggressive form of skin cancer, with curative therapy still limited to early stages. During incidences and mortality rates, as well as the high propensity for metastasis underscore the urgency to identify novel effective therapeutic strategies to overcome the poor prognosis associated with advanced melanoma. FDA-approved anti-tumor antibodies (such as rituximab and trastuzumab) have made a major impact in the treatment of other malignancies, but not yet in melanoma. These antibodies induce ADCC, and also drive antigen presentation-dependent adaptive immune responses to both neoantigens and differentiation antigens. To enhance anti-tumor antibody efficacy in melanoma, here we have explored eliminating the suppressive effects of Tregs, as well as enhancing adoptive Tcell responses using Tcell agonists. Using the B16 melanoma model, we tested the combination of TAA99 antibodies (anti-TRP1) and Treg depletion (using a single dose of anti-CD25 depleting antibody), which caused a significant reduction and delay in tumor growth, associated with a reduced immunosuppressive environment and a strong CD117+ infiltrate, as shown by quantitative multiparametrical imaging. On the basis of this result, we investigated the therapeutic potential of the combination of TAA99 antibodies and anti-CD17 (14-18B) agonist antibodies in the B16 model of melanoma. Combination treatment resulted in a rapid and significant reduction of tumor growth with strikingly durable complete responses. Our data suggest that the elimination of immunosuppressive signals and the enhancement of stimulatory pathways enhanced the vaccinal effect of TAA99 anti-tumor antibodies in melanoma. Furthermore, the combination of novel therapeutics including Treg depleting antibodies (such as mogamulizumab) or Tcell agonists (such as urelumab or uttolumab) with anti-tumor antibodies offers the possibility to expand the arsenal of immunotherapeutic approaches, including checkpoint inhibitors, currently in the clinic for melanoma.

023 The vitamin D3 analog, maxacalcitol, ameliorates imiquimod induced murine psoriasisiform skin inflammation by inducing regulatory T cells and downregulating Th17 responses
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Pсорiasis is a Th1/Th17-mediated, chronic inflammatory dermatosis, which is commonly treated with topical steroids and vitamin D3 analogs (VD3As). In this study, we compared the effects of a VD3A maxacalcitol and betamethasone valerate (BV) steroid lotion on topical imiquimod (IMQ)-induced murine psoriasisiform skin inflammation, and inflammation induced by intradermal IL-23 injection into murine ear skin. While both treatments downregulated the mRNA levels of IL-17A/IF-17, IL-22, IL-12p40, TNF-α and IL-6, only maxacalcitol downregulated IL-2p19 expression. A significant increase of Fosp3+ T cell infiltration was noted in IMQ-induced psoriasisiform skin treated with maxacalcitol, which is associated with increased IL-6 expression. Adopted T cells (CD4+CD25+ Treg) isolated from the inginal lymph nodes of donor mice treated with maxacalcitol improved IMQ-induced psoriasisiform inflammation clinically and histopathologically, showing reduced mRNA expression levels of IL-17A/IF-17, IL-22, IL-23p19, IL-12p40 and IL-6, with increased IL-10 expression, compared to the recipients of CD4+CD25+ Treg from BV-treated donor groups. These results indicate that maxacalcitol ameliorates psoriasisiform skin inflammation by inducing functional CD4+CD25+ Tregs and suppressing Th17 responses.

024 Single cell analysis reveals the autoantigen-reactive B cell cytokine production in systemic sclerosis
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Systemic sclerosis (SSc) is a connective tissue disease with an autoimmune background. Although the mechanism of SSc remains unknown, B cells are considered to play crucial roles. The autoreactive B cells have ability to produce several cytokines independent of an antibody producing function. However, the role of autoactive B cells remain unclear because number of autoactive B cells is too small to study their functions directly. In this study, we investigated the role of autoactive B cells directly using our original micro fluidic ELISA system. Methods: In this study, our medical-engineering cooperation established micro fluidic ELISA system, which integrates immunoassay into a microchip in order to detect extremely small amounts of analytes and can study single autoactive B cells. After topo isomerase (topo) I-specific B cells were purified from topo I and complete Freund's adjuvant-extimated B cells from topo I-immunized mice. Using a microfluidic ELISA system, we showed extremely small amounts of analytes and can study single autoactive B cells. By contrast, adoptive transferred of high affinity B cells from topo I-immunized mice exacerbated fibrosis. Conclusion: These results demonstrate that topo I-specific B cells were purified from topo I and complete Freund's adjuvant-extimated B cells from topo I-immunized mice. Using a microfluidic-ELISA system, which integrates immunoassay into a microchip in order to detect extremely small amounts of analytes and can study single autoactive B cells. After topo isomerase (topo) I-specific B cells were purified from topo I and complete Freund's adjuvant-extimated B cells from topo I-immunized mice exacerbated fibrosis. Conclusion: These results demonstrate that topo I-specific B cells were purified from topo I and complete Freund's adjuvant-extimated B cells from topo I-immunized mice exacerbated fibrosis.