**019**

Functional changes in Langerhans cells (LCs) may partially explain alterations in skin immunity associated with aging. While epidermal LCs were originally seen as potent APCs for initiation of antigen-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 with T cells responsive to a fragment of chicken ovalbumin, 10 µM COVA33-350 and 10 µM COVA32-129). Supernatants were collected at 48 hrs and IL-4, IL-6, IL-17, IL-22, and IFN-gamma concentrations assayed by ELISA. IL-4, IL-8, IL-17 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 IFN-gamma 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-4, IL-17A and IL-22 responses. These results may suggest a mechanism for the observed loss of immune responses with aging.

**020**

Prior sun exposure and skin-specific auto-antibodies are associated with skin immunity in systemic Lupus Erythematosus

Takafumi Yoshizaki,1 Tetsufumi Okada,2 Masahiro Nakamura,1 Tatsuro Hayashi,1 Akira Kishimoto,3 Setsumi Yoshida2, and Toshiaki Nishimura1

Department of Dermatology, Showa University School of Medicine, Tokyo, Japan, 1Department of Biochemistry, School of Pharmaceutical Sciences, Showa University, Yokohama, Japan, and 2Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown etiology characterized by multisystem inflammation. The prevalence of human psoriasis decreases with advanced age. The incidence 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 adaptive TcEl responses using TcEl agonists. Using the B16 melanoma model, we tested the combination of TAA99 antibodies (anti-TRP1) and Treg depletion (using a single dose of anti-CD35 depleting antibody) which caused a significant reduction in tumor burden and a longer survival of tumor-bearing mice. These results indicate that the combination of TAA99 antibodies and Treg depletion has the potential to improve the efficacy of TAA99 antibodies in the treatment of melanoma.

**021**

IL-17RA and not IL-17RE is required for IL-17C-mediated psoriasisiform inflammation

St. Georgiades1, F. Pitz1, N. K第一名chi1, Y. Zhou2, L. Clarnette1, and L. Bendayan1

1Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Skin inflammation, IL-17C+ mice were mated with IL-17RE-deficient (KO) mice and skin examined. By binding IL-17RA and IL-17RE. Transgenic mice overexpressing keratinocyte-IL-17C (IL-17C+) developed a psoriasiform skin phenotype. To explore the mechanisms underlying IL-17C-mediated inflammation, we generated IL-17C+ mice reared with IL-17RE-deficient (KO) mice and skin examined. IL-17C+IL-17REKO mice had similar acanthosis (39±3 vs. 45±3mm), CD4+ (14±2 vs.14±2) and CD8+ (2±1 vs. 3±1) T cell numbers as IL-17C+ mice (n=8; p>0.1). Moreover, acanthosis and T cells did not change in IL-17C+ mice transplanted with bone marrow from CD4-overexpressing IL-17RE mice vs. control mice (n=6-9). These results suggest that IL-17C signaling is IL-17RE dependent. Next, IL-17C+IL-17REKO mice were treated with anti-IL-17A antibody, which significantly decreased in acanthosis were observed. (18±3 vs. 39±3; p=0.006) suggesting that IL-17C+IL-17RA signaling is critical for skin inflammation. We have previously demonstrated in the KC-Tie2 psoriasis mouse model, a 15-fold increase in skin IL-17A and IL-17C (P<0.05) and that anti-IL-17A antibody treatment improves acanthosis and decreases CD4+ T cells vs. IgG control (36±5 vs. 22±3; v = 5.34±4; and 14±2 vs. 12±3 vs. 28±1, respectively; P<0.05; n=5-11; gplp). IL-17RA inhibition increased acanthosis before than IL-17A inhibition (P=0.02), suggesting that both IL-17C and IL-17A signaling through IL-17RA are critical. Indeed, genetic deletion of IL-17C from KC-Tie2 mice decreased acanthosis (14±1 vs. 43±4, n=8; gplp 0.001) and skin CD4+ T cells (8±1 vs. 19±2; P < 0.001) compared to KC-Tie2 mice. Interestingly, IL-17A modulation (v+ in IL-17C+) mice using anti-IL-17A antibodies, transplantsing IL-17AKo bone marrow of introducing IL-17A intradurally, 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-17RE independent, IL-17RA dependent manner and that a tiered balance between IL-17C- and IL-17RA signaling may be critical for dictating levels of inflammation.

**022**

Enhancing the vaccinal effect of anti-tumor antibodies in melanoma

R Perez-Lorenzo, AM Christiano and R Cynes

Columbia University, New York, NY

Melanoma is the most aggressive form of skin cancer, with curative therapy still limited to early stage cases. The incidence 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 adaptive TcEl responses using TcEl agonists. Using the B16 melanoma model, we tested the combination of TAA99 antibodies (anti-TRP1) and Treg depletion (using a single dose of anti-CD35 depleting antibody) which caused a significant reduction in tumor burden and a longer survival of tumor-bearing mice. These results indicate that the combination of TAA99 antibodies and Treg depletion has the potential to improve the efficacy of TAA99 antibodies in the treatment of melanoma.

**023**

The vitamin D3 analog, macacalcitol, ameliorates imiquimod induced murine psoriasisiform skin inflammation by inducing regulatory T cells and downregulating Th17 responses

C.S. Hsu1, T. Shimizu1, T. Sugaya2, T. Kadono2, S. Sato3, and S. Watanabe1

1Department of Dermatology, University of Tokyo, Tokyo, Japan and 2Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan

Vitamin D3 has been studied for its potential therapeutic effects in psoriasis, but its use is limited by its high incidence and mortality rates, as well as the high propensity for metastasis. The incidence 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 adaptive TcEl responses using TcEl agonists. Using the B16 melanoma model, we tested the combination of TAA99 antibodies (anti-TRP1) and Treg depletion (using a single dose of anti-CD35 depleting antibody) which caused a significant reduction in tumor burden and a longer survival of tumor-bearing mice. These results indicate that the combination of TAA99 antibodies and Treg depletion has the potential to improve the efficacy of TAA99 antibodies in the treatment of melanoma.

**024**

Single cell analysis reveals the autoantigen-reactive B cell cytokine production in systemischen sclerosis

A Yoshizaki1, T Fukasawa2, S Ebata2, T Kitamori2 and S Sato3 1Department of Dermatology, University of Tokyo Graduate School of Medicine, Tokyo, Japan and 2The University of Tokyo, Japan

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 immune producing function. However, the role of autoreactive B cells remain unclear, because number of autoreactive B cells is too small to study their functions directly. In this study, we investigated the role of autoreactive 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 autoreactive B cells. After topo-isomerase (topo) I-specific B cells were purified from topo I-immunized mice and complete Freund’s adjuvant-injected mice, single B cells. Then, single B cells were cultured in vitro, and the cytokine production were assessed. We also studied autoreactive B cells from SSc patients. Results: High affinity topo I-specific B cells obtained from SSc model mice produced higher amount of IL-6 compared with low affinity B cells. Furthermore, IL-10 production of high affinity cells was lower than those of low affinity cells. Similar results were obtained in SSc patients. Adptive transfer of low affinity B cells from 7 days after topo I-immunized mice inhibited skin and lung fibrosis in SSc model mice. By contrast, adoptive transferred of high affinity B cells from 7 days after topo I-immunized mice exacerbated fibrosis. Conclusion: These results suggest that the B cell cytokine production is affected by B cell autoantigen affinity. Furthermore, alteration of autoantigen-reactive B cell cytokine production can play crucial role in SSc development and progression.