001 \textbf{Biasing of the outcome of antigen (Ag) presentation by calcitonin gene-related peptide (CGRP)-exposed endothelial cells (ECs) requires CGRP-induced expression of low-density lipoprotein receptor (LDLR).} \textit{THS} \textit{Cell} 1983;4:101–106.

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Exposure of cultured microvascular ECs to CGRP endows them with the ability, acting as bystanders, to bias the outcome of Langerhan cell (LC) Ag presentation to T cells away from the Th1 pole and toward the Th17 pole. IL-6 production by ECs mediates much of this effect and, accordingly, CGRP treatment of ECs from IL-6 knock-out mice failed to bias the outcome of Ag presentation. In this regard, pre-exposure of LCs to IL-6, but not pre-exposure of T cells, biased the outcome of Ag presentation in this manner. Exposure of LCs to anti-CD126 antibody bodies before and during IL-6 treatment inhibited much of this effect, indicating that the IL-6 receptor α chain on LCs is involved in this process. Also, exposure of LCs to IL-6 upregulated LC IL-6 production. To examine if IL-6 trans-presentation by CD126 on LCs to T cells is responsible for these observations, cultures were set up as follows: (1) BALB/c LCs were treated for 3 hrs with IL-6, then washed x 4 and co-cultured with DO11.10 T cells (which spontaneously respond to CD3 and anti-CD28 antibodies). (2) The group was set up identically except that LCs were pretreated with medium alone. (3) Cultures were set up identically except that LCs were not pretreated but, rather, responding T cells were pretreated with a CD126-ΔIL-4 chimeric molecule and washed x 4 prior to setting up cultures. After 48 hrs, supernatants (SUPs) were harvested and cytokine content quantified by ELISA. 0.0001. These results strongly indicate that the effect of IL-6 treatment of LCs on biasing the outcome of Ag presentation results from trans-presentation of IL-6 by LCs to responding T cells.

003 \textbf{In vitro genetic reprogramming increases MHC-I expression and ameliorates resistance to an antitumor immune response in Merkel cell carcinoma} \textit{K. Ludy}, J. Green, S. Tzeng, and J. Sunshine \textit{Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, United States and 2 Dermatology, Johns Hopkins University, Baltimore, Maryland, United States}

Merkel cell carcinoma (MCC) is a rare but aggressive skin cancer with a certain number of patients unresponsive to immune checkpoint inhibitors (ICIs). A primary resistance mechanism driving ICI resistance in MCC is the expression of the immune checkpoint receptor (ICRH). Low expression of HLA class I (MHC-I) expression, thereby limiting cytotoxic cellular immune response. Assessment of three patient-derived MCC cell lines, MCC11, MCC26, and UISO, demonstrated low baseline MHC-I expression in 2 of the 3 cell lines (MCC11 and UISO), with higher expression in MCC26. We used biodegradable polymeric nanoparticles based on poly(beta-amino ester) to co-deliver DNA plasmids encoding a co-stimulatory molecule (4-1BBL) and a lentiviral vector (Lenti-CD8a) into the cells. This enabled the cells to interact productively with cytotoxic lymphocytes and elicit a targeted anti-tumor immune response. Following nanoparticle administration, 22, 12, and 42% of MCC11, MCC26, and UISO cells expressed surface-bound 4-1BBL and induced CD8+ T cell activation. Following nanoparticle transfection with 4-1BBL and IL-12, however, MCC11, MCC26, and UISO cell cultures had 100%, 42%, and 86-fold increased IFNγ production and 3.6-, 1.6-, and 2.8-fold CD8+ T-cell expansion, respectively, as well as significant cancer immune response. In this regard, pre-exposure of LCs to IL-6, but not pre-exposure of T cells, biased the outcome of Ag presentation in this manner. Exposure of LCs to anti-CD126 antibody bodies before and during IL-6 treatment inhibited much of this effect, indicating that the IL-6 receptor α chain on LCs is involved in this process. Also, exposure of LCs to IL-6 upregulated LC IL-6 production. To examine if IL-6 trans-presentation by CD126 on LCs to T cells is responsible for these observations, cultures were set up as follows: (1) BALB/c LCs were treated for 3 hrs with IL-6, then washed x 4 and co-cultured with DO11.10 T cells (which spontaneously respond to CD3 and anti-CD28 antibodies). (2) The group was set up identically except that LCs were pretreated with medium alone. (3) Cultures were set up identically except that LCs were not pretreated but, rather, responding T cells were pretreated with a CD126-ΔIL-4 chimeric molecule and washed x 4 prior to setting up cultures. After 48 hrs, supernatants (SUPs) were harvested and cytokine content quantified by ELISA.

004 \textbf{Immune regulatory roles of IFN$\gamma$ in skin disease} \textit{M. Mukai}, H. Takahashi and M. Amagai \textit{1 Dermatology, Keio University School of Medicine, Tokyo, Japan and 2 Laboratory for Skin Homeostasis, RIKEN IMS, Yokohama, Japan}

IFN$\gamma$ is one of the most characterized pro-inflammatory cytokines produced by type 1 T helper (Th1) cells and crucial for the defense against viral infections and autoimmune disease development. We previously established Th1-mediated interface dermatitis model that was induced by adoptive transfer of IL-$\gamma$-expressing TCR transgenic (H1) CD4$^+$ T cells into Rag2$^{-/-}$ mice. In this model, H1 T cells directly infiltrated into the epidermis and damaged keratinocytes, inducing interface dermatitis. In addition, pivotal pathogenic roles of IFNγ produced from H1 T cells were elucidated since IFNγ-H1 T cells did not cause interface dermatitis after transfer into Rag2$^{-/-}$ mice. In this study, we aimed to further investigate the roles of IFNγ signaling in the dermatitis by using IFNγ receptor (IFNγR) mice. When naive IFNγR$^{-/-}$ CD4$^+$ T cells were stimulated in vitro by anti-CD3 and anti-CD28 antibodies with IL-12, Th1 differentiation was suppressed compared to WT T cells. (6.7 ± 0.79% vs 1.2 ± 0.35%, P < 0.002). Adoptive transfer of H1-IFNγR$^{-/-}$ T cells into Rag2$^{-/-}$ did not cause the dermatitis. On the other hand, when H1 T cells were transferred into IFNγR$^{-/-}$ Rag2$^{-/-}$ mice (n = 3), the dermatitis unexpectedly appeared earlier with more severe phenotype than when Rag2$^{-/-}$ mice were used as recipients (n = 3) (clinical score, 22 ± 2.9% vs 4 ± 1.3%, P < 0.001). Flow cytometric analysis revealed that IFNγ-producing H1 cells were more abundant in the lesional skin of IFNγR$^{-/-}$-Rag2$^{-/-}$ recipients, compared to Rag2$^{-/-}$ recipients (36.3 ± 0.55% vs 13 ± 0.66%, P < 0.001). These results indicated that IFNγ signaling in H1 T cells is indispensable for inducing interface dermatitis, but the signal in non-T and B cell population present in Rag2$^{-/-}$ mice functions as an immunoregulatory pathway. Thus, IFNγ signaling exerts not only pro-inflammatory but also anti-inflammatory actions depending on the types of signal-accepting cells.

006 \textbf{Opin expression in human Langerhans cell-like cell line, ELD-1} T. Xie, Y. Lan, Y. Wang, Z. Liu and H. Lu \textit{Department of dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China}

Background: Langerhans cell (LCs) is the most powerful antigen-presenting cells (APCs), which plays a vital role in inducing and maintaining the primary immune response. Opins play an important role in modulating vision and maintaining ocular health. A recent study indicated that more than 40% of other patients and ours have shown that opin expression in human skin cells (keratinocytes, Melanocytes, Fibroblasts) and performs light and no light mediated physiological functions. However, the expression and its function of Langerhans cells have not been reported. Objective: To determine the mRNA and protein expression of opin in human Langerhans cell-like cell line, ELD-1. Methods: ELD-1 was cultured in 1640 medium supplemented with 10% FBS. The mRNA level and protein content of opin in ELD-1 were detected by quantitative real-time PCR and western blotting analysis respectively. The expression of opin in ELD-1 was analyzed by the immunofluorescence technique under the fluorescent microscope and the confocal microscope in vitro. Results: Immunofluorescence analysis demonstrated that in ELD-1, the expression of Opin1-SW, Opin2, Opin3, Opin4 and Opin5 mRNA and protein are detected in ELD-1 and opin3 mRNA and protein are significantly more abundant than other opins (p < 0.05). The results were consistent with that of opin protein by western blot analysis. Conclusion: Our study is the first report on the expression of opins in ELD-1, and opin may play an important role in LCs.