031 Inducible skin-associated lymphoid tissue (iSALT) is detected in the scalp treated with topical immunotherapy for alopecia areata
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The lymphoid tissue structure that allows the interaction between T cells, B cells and antigen-presenting dendritic cells (DCs) on a matrix made up by stromal cells. Such organized structures can also be formed in tertiary lymphoid organs (TLOs) at sites of chronic immune responses. These structures have been named according to their anatomical site, such as inducible bronchus associated lymphoid tissue (iSALT) and mucosa associated lymphoid tissue (MAIT). As similar structure in the skin, Streilein proposed a concept of skin-associated lymphoid tissue (SALT). Recently, through the detailed examination of the elicitation phase of contact hypersensitivity (CHS) as a murine model of contact dermatitis, we have confirmed sequentinal dendritic and T cells clustering in the dermal post-capillary venule, and termed this structure inducible SALT (iSALT). However, it remains unknown whether iSALT exist in human skin. Furthermore, the contributions of B cells to iSALT have not been observed in CHS response yet. In addition, whether iSALT exist in human skin. Furthermore, the contributions of B cells to iSALT have not been observed in CHS response yet. To address these issues, we focused on the topical immunotherapy for alopecia areata, which is one of the most efficient therapies of AA and induces chronic delayed-type hypersensitivity responses. In this study, we have performed immunohistochemistry in the skin section obtained from AA patients treated with topical immunotherapy to detect iSALT in human skin. As a result, immunohistochemistry revealed tightly packed infiltrations of numerous T cells, B cells and DCs in the dermal perrivascular areas and around tufted papillae. These results suggested the possibility that iSALT plays an essential role in topical immunotherapy for alopecia areata.

032 Bach2 suppresses tumor immunity by repressing effector function-related gene in CD8+ T cells
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Bach2 is a transcription repressor which binds to Maf-related elements (MAREs). Bach2 plays essential roles in B cell development, immunoglobulin class-switch recombination and somatic hypermutation of immunoglobulin encoding genes. Bach2 is also required for development of effector T cells and regulatory T cells. These findings suggest that Bach2 plays important roles in development, differentiation and functions of various immune cells. We speculated that the deficiency of Bach2 would result in an altered immune response in tumor rejection. A subcutaneous transplantation model revealed that the tumor transplanted into the Bach2 KO mice grew more slowly than the wild-type (WT) mice. These observations suggested that tumor immunity in the Bach2 KO mice was upregulated. The flowcytometry analysis revealed that the abundance of CD8+ T cells increased up to the tumors in Bach2 KO mice than WT mice. A cell trace violet (CTV) assay revealed that the Bach2 KO CD8+ T cells exhibited stronger cytotoxicity against B16F10 than the WT cells in vitro. The expression levels of GzmB and Ifnγ were higher in Bach2 KO CD8+ T cells than WT cells. An electro-phoretic mobility-shift assay revealed that Bach2 bound to the MARE-like sequence of Fast and Czmb. The binding of Bach2 to the MARE-like sequence of Czmb required the hetero- dimer formation with MafK. An immunofluorescent staining revealed that Bach2 was excluded into cytoplasmic regions from nuclear regions after TCR stimulation. These results suggest that Bach2 directly represses a set of effector genes and localizations change of Bach2 is important for the acquisition of effector function in CD8+ T cells.

033 Semaphorin 4D enhances antibody production in bullous pemphigoid
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Autoantibodies produced by plasmacytoid/activated B cells against skin basement membrane zone is a vital but not well-established mechanism in the development of bullous pemphigoid (BP). Semaphorin 4D (Sema4D) can promote B cell activation and enhance its capacity of antibody production. We sought to illustrate the implication of Sema4D in BP that facilitates B cell activation and antibody production. In our study, soluble Sema4D (Sema4D) levels in serum and blister fluid were analyzed by enzyme-linked immunosorbent assay. Immunohistochemical staining of Sema4D was performed on BP lesional tissues. CD100 expressions on membrane of immune cells in BP lesions and peripheral blood were detected by flow cytometry. Anti-BP180 antibody titers were evaluated in the supernatant of Sema4D-treated or untreated murine kerinocytes. We also employed the ELISA assay to detect the sera levels of Sema4D in both serum and blister fluid of BP patients were correlated with BP180 antibody titers and disease activities. Sema4D-expressing cells were accumulated in BP subepidermal blister as well. The expression of membrane CD100 on granulocytes rather than lymphocytes decreased to different extents in the acute phase compared with those in normal controls, and this decline almost recovered in the stable phase. In vitro, incubation of Sema4D with BP-lesional keratinocytes resulted in significantly higher levels of anti-BP180 antibody productions. We demonstrated that Sema4D derived from lesional peripheral granulocytes. We also confirmed that the expression of estrogen receptor α and β in keratinocytes and DCs using flow cytometry analysis. Taken together, these results suggest that estradiol plays protective roles in imiquimod-induced murine psoriatic dermatitis through cross-regulation of keratinocyte activation.

034 Estradiol plays regulatory roles in an imiquimod-induced murine psoriatic dermatitis through down-regulation of keratinocyte activation
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It has been reported that psoriasis symptoms have improved during pregnancy, while deteriorated after menopause, suggesting protective roles of estradiol in the development of psoriasis. In addition, the severity of psoriasis tends to be higher in men than in women in Asian countries. However, the specific mechanisms of estradiol regulating the development of psoriasis remain largely unclear. To evaluate the potential roles of estradiol on the development of psoriasis, we firstly subjected ovarectomized-female mice to an imiquimod-induced murine psoriasis model with or without systemic estradiol administration. Mice treated with estradiol exhibited significantly attenuated dermal edema, inflammatory cell infiltration and epidermal hyperplasia when compared to vehicle-treated mice. The mRNA expressions of keratinocytes-derived cytokines, such as IL-23, IL-17 and IL-1β, were significantly reduced. Moreover, the expression of various cytokines (such as IL-23p19 and IL-12/23p40) after imiquimod treatment were significantly impaired by treatment with estradiol, suggesting that estradiol exerts regulatory roles on psoriatic dermatitis by suppressing keratinocyte expression from keratinocytes and/or DCs. In vitro, estradiol directly down-regulated the mRNA induction of various cytokines (including IL-24 and CXCL1) in primary murine keratinocytes and normal human epidermal keratinocytes stimulated with aldeara and TNF-α. On the other hand, estradiol did not suppress the induction of IL-23p19 and IL-12/23p40 in imiquimod-stimulated bovine marrow-derived DCs. We also confirmed that the expression of estrogen receptor α and β in keratinocytes and DCs using flow cytometry analysis. Taken together, these results suggest that estradiol plays protective roles in imiquimod-induced murine psoriatic dermatitis via inhibiting the production of inflammatory mediators by keratinocytes.

035 Temporally controlled B cell depletion with universal chimeric antigen receptor (CAR) T cells for pemphigus vulgaris (PV) therapy
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Therapy of PV and most autoimmune diseases relies on chronic immunosuppression, which results in significant morbidity and mortality. Complete but transient B cell depletion should cure PV, since autoreactive clones do not recur upon regeneration of the B cell repertoire. In this context, genetically engineered CAR T cells (CAR-Ts) have emerged as the most potent means to achieve total B cell depletion. For autoimmune disease therapy, temporal control of CAR cytotoxicity is necessary to prevent lasting immunosuppression. Here, we validate 3 novel strategies to control CAR-T survival and function. We combined a B cell targeting CAR with an inducible caspase 9 suicide gene (iCAR), a reverse (constitutively active) suicide gene (revCAR), or a molecular on-switch that permits CAR surface expression (onCAR). iCAR, revCAR and onCAR-Ts showed potent and specific in vitro killing equivalent to conventional CAR-Ts that have proven successful in clinical trials (n<0.05). The depletion of the respective immune system resulted in rapid in vitro depletion of >95% of scAR and revCAR-Ts and reversible loss of >95% CAR surface expression in onCAR-Ts, indicating the feasibility of this approach. In an in vivo model, depletion of CAR-Ts was observed as early as 6, p<0.01 compared to vehicle treated mice, while preserving their efficacy before suicide gene activation (n=6, p<0.001). Similarly, revCAR-Ts showed complete loss of leukemia control in vivo in the absence of a suicide gene (n=8, p<0.01), indicating their complete functional depletion on vivo compared to non-transduced control T cells (n=5, p<0.23). Finally, to allow universal (allergic) CAR-T therapy, we used CRISPR/Cas genome editing to disrupt endogenous T cell receptor (TCR) function. We generated humanized allogeneic BLT mouse model (n=8, p<0.05). Suicide gene activation resulted in complete in vivo depletion of scAR-Ts compared to pre-treatment (p<0.05). In summary, our in vivo data support 3 novel strategies to regulate CAR function, which in combination with universal T cells, provide a platform for effective, large-scale applications in PV and other autoimmune diseases.