001 IL-17A induces heterogeneous macrophages, and it does not alter the effects of lipopolysaccharides on macrophage activation in the skin of mice
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Macrophages are central to inflammatory response and become polarized toward the M1 or M2 states upon activation by immunostimulants. In this study, we investigated the effects of lipopolysaccharides (LPS) and interleukin (IL)-17A on the activation of macrophages in vivo in mouse skin. We examined whether macrophages are activated in the skin of imiquimod (IMQ)-treated mice, a model for IL-17A-induced psoriasis-like skin inflammation, and flaxyl-tail (Flgft) mice, a model for IL-17A-induced chronic atopic dermatitis-like skin inflammation. LPS and IL-17A independently increased the expression levels of iNOS, CXCR1, CD206, and phospho-STAT3 proteins in the skin of Flgft mice, suggesting that macrophages to change of IMQ-treated and Flgft mice. IL-17A neutralization increased the expressions of iNOS and phospho-STAT3 in the skin of IMQ-treated mice and Flgft mice, while IL-17A neutralization decreased the expression of iNOS and phospho-STAT3 in the IMQ-treated skin, but it decreased the expressions of CD206 and phospho-STAT3 proteins in the skin of Flgft mice, suggesting that macrophages to change from the M2 to the M1 state in the skin of these mice. These results suggest that IL-17A is involved in the activation of macrophages that are in the process of adopting the heterogeneous profiles of both the M1 and M2 states.

002 Detection of serum autoantibodies to extracellular matrix protein 1 (ECM1) and relevant abnormal expression of hemidesmosomal antigens in lichen sclerosus
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Lichen sclerosus (LS) is an acquired inflammatory dermatosis that mainly affects anogenital area. The presence of serum IgG autoantibodies directed against ECM1 was demonstrable in a substantial number of patients; however, an ordinary indirect immunofluorescence (IF) procedure mostly yields negative results, and it remains uncertain as to whether the serum anti-ECM1 antibody is accessible to the in vivo native antigen in the skin and plays a pathogenic role in LS. This study aims to address the diagnostic utility of laser scanning confocal laser scanning microscopy (CLSM) system for detecting anti-ECM1 antibody in LS sera and expression profiles of structural proteins in dermo-epidermal junction/blood vessels of the LS lesional skin. Serum from patients with LS (n=13), 1 male and 12 female) were analyzed by indirect IF staining on CLSM and dot blotting using recombinant ECM1 protein. Paraformaldehyde and frozen sections from the LS lesional skin were immunostained for a panel of antibodies with reactive with hemidesmosomal antigens. On CLSM, almost all LS sera (12/13, 92.3%) exhibited increased immunoreactivity in the lower epidermis and dermal vessel walls of normal skin substrate, consistent with the staining pattern of affinity-purified IgG from LS sera and rabbit anti-ECM1 antibody. All 13 LS skin sera that had immunopositivity on either CLSM or dot blotting were immunostained for lamina densa (COL IV) and sublamina densa antigens (COL VII), demonstrating an irregular thickness of dermal vessel walls and basement membrane, but showed unchanged immunolabelling of other hemidesmosomal antigens. Indirect IF-CLSM can be a reliable tool for detection of anti-ECM1 antibodies in LS sera, and revealed altered expression of particular hemidesmosomal antigen, one of which was co-localized with ECM1. Our results suggest that in vivo bound anti-ECM1 antibody may be a pathogenic axis to establish the LS pathology.

003 The histological and immunological characterization of fibrillar-type dermatitis herpetiformis
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Dermatitis herpetiformis (DH) is a pruritic papulovesicular disease that is relatively common in Caucasian populations. It is characterized by granular IgA deposition in the dermal epidermal junction and gluten-sensitive enteropathy (GSE) associated with the HLA-DQ2/DQ8 haplotype. In contrast, DH is rare in Japan and we occasionally experience Japanese DH patients who show several unique features, including a high frequency of granular IgA deposition in the dermal epidermal junction, the rare occurrence of the HLA-DQ2/DQ8 haplotype. In these patients, IgA antibodies to epidermal transglutaminase (tTG) are detected, but IgA antibodies to tissue transglutaminase (tTG) are not. We refer to the conditions in Japanese individuals as fibrillar-type DH, while the typical DH that is observed in Caucasian individuals is referred to as granular-type DH. We performed a histological and immunological examinations using serum and skin tissue specimens obtained from typical fibrillar-type DH patients. An immunohistochemical examination showed a high frequency of fibrillar IgA deposition in the papillary dermis, as reported previously, and the IgA signals were well co-localized with those of fibrillin-1, which is a component contained in microfibril bundles. Furthermore, deposits of IL-4 and IL-5 were observed in the papillary dermis of the skin lesions of fibrillar-type DH. These suggest that Th2-type cytokines, including IL-4, IL-5 and IL-13, as well as eosinophils were elevated in patients with fibrillar-type DH. These suggest that Th2-type cytokines may be important in the development of the skin lesions of fibrillar-type DH. Taken together, we hypothesize that the pathogenesis of fibrillar-type DH patients might differ from that of typical DH, which is observed in the Caucasian population and which is dependent on gluten.

004 T cell specific microRNA-155 regulates the immune landscape of B16F10 melanoma
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MicroRNA-155 (miR-155) has recently been shown to regulate anti-tumor immune responses. However, its specific functions within distinct immune cell types during this process have not been delineated utilizing conditional knockout mouse models. In recently published work, we discovered a role for miR-155 expression within the T cell compartment during the immune response to syngeneic B16F10 mouse melanoma tumors. We found that miR-155 expression within T cells is required to limit syngeneic tumor growth and promote interferon gamma (IFNγ) production within the tumor microenvironment. Consequently, miR-155 expression by T cells was necessary for proper tumor associated macrophage (TAM) expression of interferon gamma inducible genes. We also found immune checkpoint blockade (ICB) antibodies to program death ligand 1 (PD-L1) and cytotoxic T lymphocyte associated protein 4 (CTLA-4) restored antitumor immunity in miR-155 T cell conditional knockout mice by rescuing T cell IFNγ expression, TAM activation, and T cell expression of multiple activation and effector genes expressed by tumor infiltrating CD8+ T cells and CD4+ T cells. ICB partially restored expression of several derepressed miR-155 targets in CD8+ tumor infiltrating T cells, suggesting that miR-155 and ICB regulate overlapping pathways to promote anti-tumor immunity. More recently, we performed 10X single cell sequencing of tumor infiltrating CD8+ immune cells from the tumors of miR-155 T cell conditional knockout mice and further characterized the role of miR-155 expression within T cells in the process of shaping the immune landscape of the tumor microenvironment. This work will hopefully culminate in the ability to modulate miR-155 within the T cells of cancer patients to promote anti-tumor immune responses and improve clinical outcomes.

005 Adaptive and Auto-Immunity | ABSTRACTS

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The histological and auto-immunity of malignant melanoma tumors of miR-155 T cell conditional knockout mice and further characterized the role of miR-155 expression within T cells in the process of shaping the immune landscape of the tumor microenvironment. This work will hopefully culminate in the ability to modulate miR-155 within the T cells of cancer patients to promote anti-tumor immune responses and improve clinical outcomes.

006 Tumor-infiltrating CD5+ regulatory B cells suppress melanoma tumor immunity via inhibiting cytokine production of tumor-infiltrating CD8+ T cells
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We have previously identified 2 Breg subsets derived from CD19+ marginal zone (MZ) Bregs and CD5+ B1regs. In this study, we investigated the role of Bregs in tumor immunity. We used B cell-specific phosphatase and tensin homolog (PTEN)-deficient mice and B16F10 melanoma cells, which were subcutaneously injected into mice. We assessed melanoma growth and tumor-infiltrating lymphocytes by flow cytometry analysis. In B cell-specific PTEN-deficient mice, B cell number and Breg subset were expanded and melanoma growth was increased as compared to control mice. There was no difference in tumor-infiltrating T cell, regulatory T cell, and NK cell numbers between B cell-specific PTEN-deficient and control mice. However, in B-reg mutant and TNF-z− deficient B1regs were decreased in B-cell specific PTEN-deficient mice. In regard to Granzyme B secretion by tumor-infiltrating NK cells, there was no difference between both mice. The numbers of tumor-infiltrating Bregs were significantly increased in B cell-specific PTEN-deficient mice. More than 20% of the tumor-infiltrating B cells consisted of Bregs in both B cell-specific PTEN-deficient mice and control mice. In addition, most of tumor-infiltrating Bregs consisted of B1regs, but not MZ Breg. Adoptive transfer of CD5+ B cells to B16F10 tumor-infiltrating mice increased melanoma growth while non-B1 cells had no effect. These results suggest that tumor-infiltrating CD5+ B1regs negatively regulate tumor immunity by reducing Th1 cytokine production of tumor-infiltrating CD8+ T cells.

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