025 Combination of breathing exercises, cold exposure, and meditation mitigate psoriasis – open label, randomized, controlled trial
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Psoriasis is a skin disease of an unknown origin. Current understanding of its pathophysiology focuses on an unbalanced immune response among the dermal and epidermal systems. In this study, we aimed to evaluate the potential of various therapies on the development of experimental psoriasis in mice. Methods: 45 male CD1 mice weighing 30 ± 10g were divided into 5 equal groups. Low-frequency cold exposure was performed using cold plates with an adjustable temperature of 0°C. Breathing exercises were performed using a specific breathing technique, and meditation was performed using the “Use the Force” technique. The effects of these therapies on the development of psoriasis were evaluated by measuring the thickness of the epidermis and the number of角化 cells. Results: The treatment groups showed a significant decrease in the thickness of the epidermis and a decrease in the number of角化 cells compared to the control group. Conclusion: The combination of breathing exercises, cold exposure, and meditation can be an effective and safe add-on therapy for psoriasis and its comorbidities.

026 Detection of rare autoreactive T cell subsets in patients with pemphigus vulgaris by the CD154 activation assay
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Analysis of T cell proliferative responses to antigen or mitogenic stimuli are based on e.g. MTT, [3H]-thymidine incorporation or titrated thymidine or BrdU labeling and the detection of secreted cytokines by ELISA or ELISPOT assay. There are inherent drawbacks of these methods rather long ex vivo expansion, stimulation protocols or the inability to distinguish specific cell populations and the assumption of importance of certain cytokines. Rapid identification and quantification of low-frequent autoreactive T cells, however, presents a main goal in autoimmune diseases such as pemphigus vulgaris (PV). In this study, we characterized the expression of intracellular CD154 as a marker for activated CD4+ T cell subsets in PV patients and healthy controls (HC) after both ex vivo polyclonal and antigen-specific stimulation. Polyclonal stimulation using PHA resulted in significantly higher CD154 T cell expression in HC compared to PV pointing at a general T cell exhaustion caused by treatment or clinical stage of disease. In contrast, upon stimulation with human desmoglein (Dsg3), the major autoantigen in PV, there were no differences in CD154 expression between CD4+ T cells from PV patients compared to HC. Furthermore, patients with active disease showed subset-specific differences in CD4+ Th as well as in CXCR5+ T follicular helper (Thf) cells characterized by increased numbers of Dsg3-specific Th17 and Th17 cells in comparison to remittent PV patients and HC suggesting a predominant involvement of IL-17-secreting T cells in acute stages of PV. In addition, intracellular cytokine staining revealed a significant increase of IL-21, a known inducer of immunoglobulin production and Th17 cell conversion, in activated CD154+ T cells. In summary, here we show that analyzing CD154 expression allows the detection of rare antigen-specific T cell subsets and the discrimination of different Dsg3-specific Th and Thf cell populations in PV patients according to their clinical disease stage.

027 2Gy low-dose total body irradiation facilitates antitumoral Th1 immune responses in tumor antigen specific Th1 cell and immune checkpoint inhibitor-based cancer immunotherapy
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CD4+ T helper cells are capable of mediating long-term antitumoral immune responses. We developed a combined immunotherapy (COMBO) using tumor antigen-specific T helper 1 cells (Tag-Th1), dual PD-1/LAG-3 immune checkpoint blockades and a low-dose total-body irradiation (TBI) of 2 Gy, that was highly effective in controlling the tumor burden of non-immunogenic B16F10 melanoma tumors xenografted into a syngeneic RIP1-Tag2 mouse model. Our main aim was to explore if and how TBI could impact the efficacy of the Tag-Th1 combinations, and to identify the different mechanisms behind such effects. We analyzed the presence of type 1 (CXCR3+CCR6-), type 2 (CXCR3 - CCR6-), type 17 (CXCR3+CCR6+) regulatory subsets in pemphigus patients and controls. We also characterized four Treg/Tfr subsets based on their chemokine receptor expression profile. Further subsets were determined by their chemokine receptor profile. In addition, we sorted Treg17.1 and Tfr2 cells. We are currently studying the functional role of these regulatory T cells in the context of pemphigus.

028 Detection of rare autoreactive T cell subsets in patients with pemphigus vulgaris by the CD154 activation assay
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Pemphigus is a severe blistering disorder of skin and mucosa characterized by autoantibodies against desmoplakin proteins of the skin. The interaction between T ( follicular) helper cells and autoreactive T cells producing IgG antibodies is seen in about 60% of patients. Here we present our experience with the role of T regulatory cells (Treg) as critical cellular checkpoints leading to tolerance or autoimmunity. Peripheral blood mononuclear cells were collected from pemphigus patients and healthy controls for flow cytometry analysis. The cells were gated based on Th1/Tfh cell surface markers and specific markers for regulatory T cells including CD25+CD127low. Further subsets were determined by their chemokine receptor profile. In addition, we sorted Treg17.1 and Tfr2 cells and analyzed their cytokine transcripts. We characterized four Treg/Tfr subsets based on their chemokine receptor expression profile. We analyzed the presence of type 1 (CXCR3CCR6), type 2 (CXCR3 CCR6), type 17 (CXCR3CCR6+) or type 17.1 (CXCR3CCR6+) regulatory subsets in pemphigus patients (n=63) and healthy individuals (n=19). We found significant higher percentages of Treg17.1 and Tfr2 cells in pemphigus patients compared to controls. Further analyses showed the expression of cytokines and transcription factors that confirmed the regulatory characteristics of the aforementioned subsets by positive expression of FOXP3 and TGFβ. We observed a common pattern also but some different expression levels of cytokines between specific cell subsets. Our flow cytometry panel allowed us to characterize Treg and Tfr cell subsets in an auto-antibody-mediated blistering skin disease, where we specifically found a dominance of regulatory cells and an increase in the ratio of Treg17.1 and Tfr2 cells. We are currently applying the functional role of these regulatory T cell subsets in cellular assays and longitudinal studies.

029 Deciphering the functionality of T regulatory subsets in pemphigus
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Pemphigus is a severe blistering disorder of skin and mucosa characterized by autoantibodies against desmoplakin proteins of the skin. The interaction between T ( follicular) helper cells and autoreactive T cells producing IgG antibodies is seen in about 60% of patients. Here we present our experience with the role of T regulatory cells (Treg) as critical cellular checkpoints leading to tolerance or autoimmunity. Peripheral blood mononuclear cells were collected from pemphigus patients and healthy controls for flow cytometry analysis. The cells were gated based on Th1/Tfh cell surface markers and specific markers for regulatory T cells including CD25+CD127low. Further subsets were determined by their chemokine receptor profile. In addition, we sorted Treg17.1 and Tfr2 cells and analyzed their cytokine transcripts. We characterized four Treg/Tfr subsets based on their chemokine receptor expression profile. We analyzed the presence of type 1 (CXCR3CCR6), type 2 (CXCR3 CCR6), type 17 (CXCR3CCR6+) or type 17.1 (CXCR3CCR6+) regulatory subsets in pemphigus patients (n=63) and healthy individuals (n=19). We found significant higher percentages of Treg17.1 and Tfr2 cells in pemphigus patients compared to controls. Further analyses showed the expression of cytokines and transcription factors that confirmed the regulatory characteristics of the aforementioned subsets by positive expression of FOXP3 and TGFβ. We observed a common pattern also but some different expression levels of cytokines between specific cell subsets. Our flow cytometry panel allowed us to characterize Treg and Tfr cell subsets in an auto-antibody-mediated blistering skin disease, where we specifically found a dominance of regulatory cells and an increase in the ratio of Treg17.1 and Tfr2 cells. We are currently applying the functional role of these regulatory T cell subsets in cellular assays and longitudinal studies.

030 Non-infective Complications of Rituximab during Treatment for Autoimmune Blistering Diseases
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Rituximab (CD20 monoclonal antibody) is considered the first-line treatment in new-onset moderate-to-severe pemphigus and/or patients who do not achieve clinical remission with systemic corticosteroids and/or immunosuppressive agents. Studies have demonstrated that Rituximab affects reactions, infections, and laboratory abnormalities to generally be the leading adverse events of rituximab treatment regardless of disease. We performed a systematic review to focus on the non-infectious complications of rituximab treatment that providers may not be aware of. Our search was performed between 22 February 2019 and 10 July 2019 using a combination of keywords. Case reports, case series, and recent reviews were included. Of 1438 patients with blistering diseases were reported, of which 300 (20.8%) had adverse effects categorized as minor and major according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Events included 14.5% were non-infectious and represented cardiovascular, gastrointestinal, neoplastic processes, cutaneous, and renal processes. Of major adverse events, 23% were neither infectious nor inflammation reactions, and included laboratory abnormalities, close monitoring of patients treated with rituximab is recommended.

031 Adaptive Immunity and Autoimmunity | ABSTRACTS

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