Different effects of combined blockade of IL-4/IL-13 and selective inhibition of IL-13 in in vitro model systems for atopic dermatitis with allergen stimulated lymphocytes

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New approaches for the treatment of atopic dermatitis (AD) have focused on the development of humanized antibodies directed against Th2 cytokines for the last few years. Dupilumab is the only antibody approved for the treatment of AD so far and blocks the IL-4Rα receptor subunit, inhibiting IL-4/IL-13 signaling pathways. Two specific anti-IL-13 antibodies have so far shown promise in phase II clinical trials. However, context of IL-4 and IL-13 have similarities, in part because they share the same receptor subunits and thus signal through similar pathways. Our work focuses on the question of whether combined blockade of IL-13 and IL-4 or selective inhibition of IL-13 have different functional effects on lymphocytes from sensitized patients with atopic dermatitis. After stimulation of mononuclear cells from the blood of patients sensitized via IgE against house dust mite or against autoregans, antigen induced proliferation and cytokine production were measured after IL-4 and IL-13 blockade. T-cell subtypes were determined and IL-lymphocytes were examined with regard to IgE production. Surprisingly, combined IL-4/IL-13 blockade led to an increase in antigen-specific growth of mononuclear cells in short-term cultures over 7 days. This effect was not caused by IL-11 inhibition alone. The investigations with long-term cultures over 3 weeks showed a suppressive effect on the growth of the antigen-specific T-cell lines by both the selective IL-11 and the combined IL-4/IL-13 blockade. Moreover, specific IL-11 treatment had an effect on IL-5 and IL-17 levels whereas ALL-4/IL-13 treatments affected IL-5 levels only. IgE monitoring showed that both forms of IL-4/IL-13 inhibition reduced in vitro IgE production in anti-CD40 plus IL-13 stimulated cells by more than 70%. Our work shows different functional effects by combined IL-4/IL-13 resp. by selective IL-11 blockade raising the question of the benefit of additional IL-4 inhibition in Th2 directed treatments of AD.

Role of hippo signaling in apoptosis of lupus keratinocytes

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The Hippo signaling pathway is a critical component in the regulation of the foilpathway. Through a kinase cascade that includes LATS1/2, TAZ and WW1C, the Hippo pathway targets YAP for phosphorylation, preventing nuclear translocation and transcriptional activity of YAP in SLE skin. We have previously developed an ex vivo model system to study the roles of YAP in lupus keratinocytes. In this study, we investigated the role of YAP in cell death and apoptosis. YAP and S6 phosphorylation in lupus keratinocytes were detected. We found that the cytoplasmic retention of YAP was associated with increased apoptosis of lupus keratinocytes. These results suggest that YAP plays a role in the regulation of cell death and apoptosis in SLE skin.

Endotypes of mucous membrane pemphigoid predict disease severity

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Mucous membrane pemphigoid (MMP) is an autoimmune blistering disease predominantly involving mucosa and is caused by antibodies directed against BP180. Collagen VII, Laminin 332, or gB3 integrin. Oral/pharyngeal lesions are the most common, but any area of mucosal epithelium may be involved. The potential long-term consequences are devastating, including blindness, airway compromise, loss of dexterity and stenoses. Despite its morbidity, there are few therapeutic options. The goal of our studies was to determine whether the severity of MMP can be resolved into distinct disease endotypes based on the autoantibody target. Seventy-one patients who met clinical, histological and immunologic criteria for MMP were enrolled in the study. The autoantibodies were determined using indirect immunofluorescence, ELISAs and immunoblotting. BP180 was the most common autoantigens, identified in 86% of cases. All patients were positive for the common PV-associated HLA genes (HLA-A*0201, -B*0801). However, the presence of autoantibodies to these targets does not fully explain disease activity. We and others have described the presence of non-Dog autoantibodies in MMP patients. To date, the scope, specificity, and particularly functional importance of non-Dog autoantibodies has not been fully defined. Our group has previously reported the discovery of a patient derived antibody (AS13) that has 74% heavy-chain homology to anti-thyroid peroxidase (TPO) antibody and 86% light-chain homology to an anti-thyroid peroxidase antibody as best alignment. While this antibody did not bind to Dog1, it did not bind to ELISA and Western Blot and did not stain intercellular regions on monkey esophagus by IF we did observe binding to a 55-60kDa protein in HaCaT keratinocyte lysates. Additionally, immunoaffinity purification of the cytoplasmic fraction of HaCaT keratinocytes but no co-localization with the cell membrane or any component thereof, including Dsg1. In order to investigate the functional role of this novel antibody and its potential to induce keratinocyte dissociation, HaCaT keratinocytes were grown to confluence and subjected to treatment with increasing concentrations of AS13, an established mouse anti-human Dsg1 antibody, and AS15. We show that while AS13 induces a strong dose-dependent dissociation of keratinocytes in vitro, the rate of fragmentation is substantially lower than that of AS15 alone. AS113 in combination with AS12, however, induces an approximately 3-fold higher fragmentation rate than AS12 alone, indicating a synergistic effect of these autoAbs in vivo. While the exact epitope target of the AS13 antibody is yet to be defined, our data suggests a functional activity of this novel patient-derived antibody in the skin with potential disease relevance.

TSS1-1: Staphylococcus aureus in bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune blistering disease that is treated with high dose immunosuppression due to lack of specific targets. Staphylococcus aureus is a commensal bacterium implicated inflammatory and autoimmune diseases because of its secretion of superantigens. We prospectively evaluated S. aureus colonization and its production of toxic shock syndrome toxin (TSS-1) in 28 new onset BP patients. Inclusion criteria were active blistering and linear basement membrane IgG/G3 or a serum ELISA > 14 for BP180 IgG. Bacterial swabs were obtained from the lesion interior, nare and unaffected skin. The sensitivity of this assay for detection of S. aureus colonization is 95%. The TSS-1 was detected in 36% of the samples, and matched skin of 28 ago- and sex-matched controls. Staphylococcal growth was assessed on blood agar, and TSS-1 production by internal S. aureus isolates and in blister fluid was evaluated by immunoblot. S. aureus colonization in BP represented 3-4 fold higher than the nare or unaffected skin from the same patients (p = 0.030) and 6-fold higher than control nare or skin (p = 0.0015). Evaluation of superantigen gene profiles using PCR indicated that 96% of BP patients are colonized with S. aureus. A superantigen gene profile survey of TSS-1+ S. aureus colonization and circulating neutralizing antibodies, measured by ELISA, did not correlate. Interestingly, S. aureus colonization was not observed in patients who had received prior antibiotics. In colonized patients with severe disease, addition of anti-staphylococcal antibiotics resulted in clinical improvement and reduced TSS-1 levels. In conclusion, we demonstrate that a single strain of TSS-1 producing S. aureus that is not evident in the general elderly population. Thus, immunosuppressive therapies should be balanced with the knowledge that S. aureus is likely to cause BP lesions and the knowledge that antibiotics may play an important therapeutic role through bacterial clearance.