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OR.73. MicroRNA 146 Activates NFkB Pathway and Possibly Modulates Intestinal Inflammation

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Introduction: MicroRNAs are non-coding RNA oligonucleotides which specifically bind to target mRNAs and leading to their degradation or inhibition of translation. However, little is known about the function of microRNAs in the filed of inflammation. We have investigated the potential role of microRNA-146 (miR-146) in the intestinal inflammation. Methods: 1. Differential expression of microRNAs in intestinal tissues of IL-10 deficient mice was examined with mirVanaTM miRNA Bioarray (Filgen). 2. Expression vectors containing a whole sequence of miR-146-a/b or each siRNA wrapped with HVJ envelope were intraperitoneally injected to mice. The protein samples obtained from the mice 48 hours after the transfection, and the phosphorylation of NFkB was examined using western blotting. 3. The survival rate were compared between two groups, 4% DSS-treated mice and control mice, both were transfected with miR146-a/b vectors. Result: 1. In colonic tissues of IL-10 deficient mice, 19 microRNAs were downregulated and 26 microRNAs including miR-146-b were upregulated more than twice. 2. NFkB phosphorylation was increased in the mice colon overexpressing either miR-146-a or -b. In contrast, each siRNA decreased phospho-NFkB expression. 3. The rate of survival was improved with the DSS-treated mice when transfecting the vectors. Conclusion: microRNA-146-a and -b activate a NFkB pathway and possibly contribute the regulation of intestinal inflammation.

OR.74. CD3-IL-2R+ Peyer's Patch Cells Respond to Microbial Stimuli, Migrate to the Lamina Propria, Secrete IL-5, and Induce IgA Production

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Objectives: We have shown that CD4-c-kit-CD3-IL-2R+ Peyer's patch (PP) cells (CD3-IL-2R+ cells) were capable of producing high levels of IL-5 and promoting IgA production by B cells. We also found that CD3-IL-2R+ cells expressed TLRs(1-4, 6-9), and responded to various TLR stimuli. Poly I:C stimulation especially induced high levels of IL-5 production, and in addition, CCR9 expression. In this study, we examined the migration properties of PP CD3-IL-2R+ cells after TLR stimulation using adoptive transfer studies, and the role of intestinal microbiota in the response of these cells using germ-free mice. Results: Adoptive transfer studies using CD45.1 and CD45.2 mice suggested that PP CD3-IL-2R+ cells migrated to LP after poly I:C stimulation. Although CD3-IL-2R+ cells were present in germ-free mice, the expression of IL-5 mRNA in these cells was dramatically lower. Conclusion: Our results taken together suggest that CD3-IL-2R+ cells sense microbial components via TLRs, migrate from PP to LP, and support intestinal IgA production through IL-5 secretion. Our studies suggest that CD3-IL-2R+ cells represent a novel subset of cells which recognize and respond to microbial components and virus infection by IL-5 production.

OR.75. Cigarette Smoke Extract Induces TSLP Expression, Leading to Th2-type Immune Responses and Airway Inflammation

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Background: Both active and passive smoking are considered to be risk factors for asthma development. However, the precise mechanisms involved remain elusive. Recently, thymic stromal lymphopoietin (TSLP) has been shown to play a key role in the development of Th2-type allergic inflammation in asthma. Objective: The aim of this study was to investigate whether there was a causal relationship between cigarette smoke exposure and TSLP expression in the lung. Methods: We examined the effects of repeated intranasal exposure of cigarette smoke extract (CSE) on TSLP mRNA and protein expression in the mouse lung by real-time PCR, Western blot, and immunohistochemistry. We also examined the effects of intranasal exposure of CSE plus ovalubumin (OVA) on Th2-type immune responses and lung pathology. Results: Repeated exposure of CSE induced TSLP mRNA and protein expression, which was inhibited by treatment with anti-oxidative N-acetylcystein (NAC) and by TNF-α receptor I (TNFRI) deficiency. In addition, the intranasal exposure of CSE simultaneously with OVA induced OVA-specific Th2-type immune responses and airway inflammation, which were inhibited by the blockade of the TSLP activity. Conclusion: CSE induced TSLP expression in the mouse lung in an oxidative stress- and TNFRI-dependent manner and, when challenged simultaneously with an antigen, CSE promoted the development of airway inflammation in association with the Th2-type immune responses. Clinical implications: The induction of TSLP by cigarette smoke may therefore play a key role in the development of asthma associated with cigarette smoke exposure.

OR.76. Transcription Factor NFATc2 Controls Apoptosis and Activation of T Cells by IL-6 in Colitis

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The functional role of T cell transcription factors such as nuclear factor of activated T cells (NFAT) in IBD is poorly understood.

The aim of this study was to analyze the role of this signal transduction pathway and its pathogenic significance in UC. Cryosesctions of UC and CD patients were analysed immunohistochemically. A significantly higher expression of NFATc2 was found in UC and CD colonic tissue compared to control specimen. Transmitted to the Th2-mediated oxazolone-induced colitis model, NFATc2-production is significantly increased in both diseases, too. NFATc2 deficient mice were analyzed in colitis model and are significantly protected against the development of intestinal inflammation compared to control mice, documented by miniendoscopy. Interestingly, cyrosections of inflamed colonic tissue displayed a higher apoptotic rate in NFATc2 deficient mice compared to control mice, which can be observed by TUNEL assays, caspase3 and Annexin V staining, as well as in lamina propria T cells. Anti-apoptotic proteins, like bcl-2 and bcl-xL were downregulated for induction of apoptosis. This observation was associated with a reduced production of IL-6, IFN-γ, IL-13 and IL-17 by mucosal T lymphocytes, tested by ELISA assays. Further studies with the oxazolone-induced colitis model showed that NFATc2 regulates IL-23/IL-17 in an indirect way. Administration of IL-6 blocked the protective effects of the NFATc2 deficiency in experimental colitis, suggesting that NFATc2 through IL-6 signal transduction plays a direct pathogenic role *in vivo*. Our data define a unique regulatory role of NFATc2 in colitis by controlling mucosal T cell activation in an IL-6 dependent manner. The examination of this signal transduction pathway emerges as a potentially new therapeutic target for inflammatory bowel diseases.