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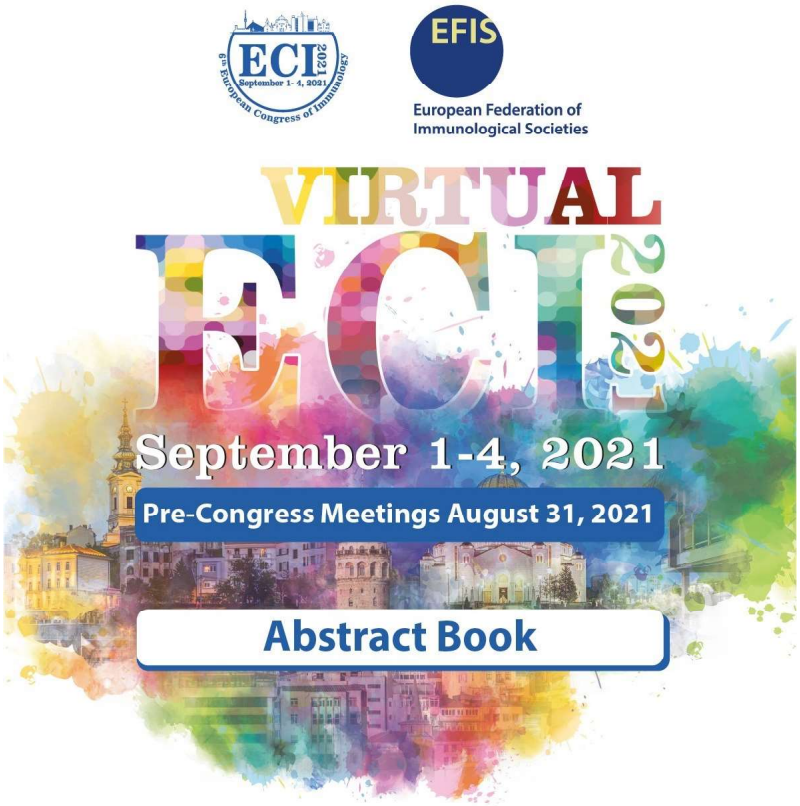

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POSTER PRESENTATIONS

P-0339

The effect of Ca2+ at a physiological concentration on primary human macrophage polarization

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Ca2+ at a physiological concentration is well known for enhancing the production of TNF-α/IFN-γ by T cells and the quantity of immune cells positive for IL-10, IL-22, IL-17, TNF-α, and IFN-γ, yet its effects on primary human macrophage polarization remain to be elucidated. Macrophages can polarize into two main classes, M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. Furthermore, M2 macrophages are divided into 4 subsets. Here, we reported the effect of Ca2+ at a physiological concentration on primary human M1, M2a, and M2c macrophages. In this study, primary human macrophages were cultured in regular RPMI (containing 0.8 mM CaCl2) or 1mM CaCl2 added RPMI which had the final concentration of 1.8 mM-. physiological concentration. The cells were differentiated into M1 with LPS+IFN-γ, M2 with IL-4, or M2c with IL-10. The M1 and M2 markers were analyzed by flow cytometry. Polarized macrophages cultured in Ca2+ at a physiological concentration demonstrated an enhanced level of cell death in a manner directly proportional to duration of the treatment. After Ca2+treatment, although the expression of the M1 markers (HLA-DRα/CD86/CD64/TNFα/CXCL10) by M1 macrophages did not change, the expression of HLA-DRα/CD86 was enhanced in M2 macrophages In addition, the expression of the M2 markers (CD206/CD200R) was decreased in M2 macrophages. Likewise, the expression of the M2 marker CD163 was decreased, while, the expression of the M1 marker CD86 was increased in M2c macrophages. In summary, our findings suggest that the macrophages tend to shift towards an M1 phenotype in the presence of Ca2+ at a physiological concentration.

Keywords: Cytokines and mediators, innate immunity, macrophage

P-0340

Soluble CEACAM1 induces suppressive Tregs by binding to CD5

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Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is an immune checkpoint regulator that controls immunity via self- or heteroligation. Soluble CEACAM1 (sCC1) is elevated in the serum of patients with obstructive and autoimmune liver diseases. Ceacam1-/- mice exhibit exacerbation and persistence of Concanavalin A hepatitis due to quantitative and qualitative regulatory T cell (Treg) impairment. T cell activation and Treg induction require the short CEACAM1 isoform (CEACAM1-S), whereas its ITIM-bearing long isoform (CEACAM1-L) limits effector responses. Recently, we identified the Treg regulator CD5 as a binding partner for sCC1. To reveal the role of the sCC1-CD5 axis in Treg-induction. sCC1 was detected in Western Blots in diseased humans and mice. sCC1 binding to CD5 was identified by LC-MS/MS. Tregs were induced with or without addition of sCC1, sCD5, or αCC1 and αCD5 antibodies. Cellular signaling (pSTAT5, Foxp3, pSmad2/3, mTOR) was analyzed in FACS and Western Blots. Induced Tregs were subjected to suppression assays. Results. sCC1 was detectable in sera of patients and mice with advanced PSC or ConA hepatitis. Interaction between CEACAM1 and CD5 was confirmed by LC-MS/MS and cell binding assays. sCC1 binding to activated T cells induced pSTAT5 and Foxp3, but reduced mTOR activation. These effects were sensitive to addition of αCD5 antibodies. sCC1-induced Tregs were capable to suppress effector cell proliferation. Addition of sCC1 to T cells supports Treg induction by acting upstream of CD5mediated mTOR inhibition. Currently, the relevance of the CEACAM1-CD5 interaction for Treg homeostasis is under validation.

Keywords: Immune regulation and therapy, autoimmunity, regulatory cells

P-0341

Understanding the molecular pathogenesis of primary autoimmune thrombocytopenic purpura. The role of transcription factor Ets-2

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In primary idiopathic/autoimmune thrombocytopenic purpura (pITP), autoreactive B and T cells initiate and sustain platelet destruction in a milieu of T helper (Th-1) and Th17 effector cell polarization, whereas regulatory Th cells (Tregs) malfunction and fail to maintain tolerance. We recently showed that in controls IL-2 is repressed in naive Th cells by the transcription factor Ets-2. In this work, we examined Ets-2 and cytokine gene expression in naive and memory Th cells (Teffs) and Tregs isolated from pITP patients and controlsto investigate the Ets-2 role in pITP pathogenesis. Blood samples were collected from 6 pITP patients and 6 age/sex-matched controls. Naive (CD4+CD45RA+CD25-) and memory (CD4+CD45RO+CD25-) Teffs and Tregs (CD4+CD25+) were isolated. Phenotypic analysis revealed increased levels of naive Teffs and decreased levels of memory Teffs and Tregs in pITP patients versus controls. Bioinformatic analysis revealed multiple Ets-2 binding sites at the promoters of Th-cell signature cytokines. In pITP naive Teffs, Ets-2 mRNA and protein synthesis were significantly lower than controls. pITP naive Teffs constitutively expressed IL-2 and IFN-γ and memory Teffs, IL-17. pITP Tregs constitutively expressed IL-2 and IFN-γ, whereas control Tregs did not constitutively express these cytokines. Compared to control, pITP Tregs constitutively expressed lower IL-10 mRNA levels. Our results suggest that Ets-2 low expression and synthesis in naive Teffs of pITP patients leads to impaired downstream events in Th cell plasticity. This manifests as high constitutive gene expression of Th1/Th17 cytokines in Teffs and abnormal cytokine gene expression in Tregs.

Keywords: Autoimmunity, epigenetic control and modulation of immunity, molecular immunology

P-0343

The influence of human endogenous retroviruses and associated transcripts on colony stimulating factor 1 (CSF1/MCSF) expression in Hodgkin lymphoma cells

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Germline infections by retroviruses during evolution caused the integration of viral DNA into the human genome, which today consists of about 8-10 % of human endogenous retroviruses (HERVs). Few HERVs have open reading frames for the formation of proteins. In varying diseases, HERVs and related long terminal repeat (LTR)-elements have been observed to affect the expression of neighboring genes. We analyzed Hodgkin lymphoma (HL) cells to find HL-associated HERV that may play a role in pathogenesis. We analyzed cDNA libraries, DNA microarrays and RNA sequencing data from HL cells. Using various computational and molecular biology approaches, we identified expressed HERV in HL cells. We discovered novel HERV-related transcripts derived from the chromosomal region directly upstream of the colony-stimulating factor 1 (CSF1/MCSF) gene. The first exon of these *T*ranscripts from *HO*dgkin *L*ymphoma c*E*lls (THOLE) belongs to the LTR8 family of HERV. We detected different THOLE-initiated CSF1 transcripts in HL cell lines. High expression of THOLE was observed only in HL cell lines. Activation of THOLE and other HERVs/LTRs with subsequent transcription of HL-associated genes like CSF1 might explain the specific gene expression profile of HL cells.

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Keywords: Cancer immunology, cytokines and mediators, molecular immunology, omics technologies, proliferative disorders