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P94 TLR7 AND TLR8 DIFFERENTIALLY ACTIVATE THE IRF AND NF-KB PATHWAYS IN SPECIFIC CELL TYPES TO PROMOTE INFLAMMATION

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Background TLR7 and TLR8 are pattern recognition receptors that reside in the endosome and are activated by ssRNA molecules. TLR7 and TLR8 participate in the anti-viral defense response but their aberrant activation has also been implicated as a driver of autoimmune diseases such as lupus. The receptors have slightly different ligand binding specificities and cellular expression patterns suggesting they have non-redundant specialized roles. How the roles of TLR7 and TLR8 differ in driving disease may be determined by which cell types express the TLRs and how they respond to activation of each.
Methods To delineate the differential effects of TLR7 or TLR8 activation we used gene expression analysis and intracellular cytokine staining to characterize changes induced by TLR specific agonists in different immune cell types. Anti-IFNR antibody treatment was also used in whole blood and lupus mice to further define which responses are a direct consequence of TLR7/8 activation and which are secondary responses driven by Type I interferon or cytokines produced subsequent to the primary response.
Results It was found that the IRF and NF-kB pathways are differentially activated downstream of the TLRs in various cell types. The differential expression and activity of TLR7 vs TLR8 was notable when comparing different DC populations, monocytes, and neutrophils. TLR7 was more biased toward activation of IRF responses while TLR8 activation resulted in stronger NF-kB signaling. The anti-IFNR antibody studies showed that inhibiting IFN activity can block secondary IFN-induced gene expression changes downstream of TLR7/8 activation, but not NF-kB-regulated genes induced directly by TLR7/8 activation at early timepoints.
Conclusions In summary, these results elucidate the ways in which TLR7 and TLR8 regulate immunity and how their activation may shape an immune response. The results also have implications for selecting strategies for treating autoimmune diseases driven by TLR7/8 activation.

P95 COSTIMULATORY MOLECULES ON CMV-SPECIFIC T-CELLS IN CMV IGG+ PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background Cytomegalovirus (CMV) infection is an uncommon but severe infection in patients with systemic lupus erythematosus (SLE) due to immunosuppressive therapy. Standard prophylaxis with antiviral agents or pre-emptive strategies to monitor viral load are not standard of care. The aim of the present study was to investigate the expression of coinhibitory molecules PD-1 and BTLA-4 on CMV specific T-cells in SLE-patients.
Methods Twenty-three SLE-patients and eight healthy controls were enrolled. Nineteen SLE patients were CMV IgG+, four were CMV IgG-. Peripheral blood was sampled and stimulated with CMV lysate, SEB or control serum in presence of anti-CD28/CD49d. After six hours of stimulation, CD154 expression was determined by flow cytometry on $CD3^+$ T-cells. The coinhibitory molecules PD-1 and BTLA were determined on activated $CD154^+CD3^+$ T-cells. Symptomatic CMV infection was defined as CMV syndrome or tissue invasive disease. Asymptomatic CMV infection was defined as detectable CMV replication in peripheral blood and absence of signs indicating CMV syndrome/tissue invasive disease.
Results PD-1 and BTLA-4 expression was not significantly different on CMV-specific $CD154^+CD3^+$ T-cells in SLE-patients as compared to healthy controls. An analysis according to the CMV serostatus revealed a tendency to a decreased proportion of $PD\text{-}1^+$ $CD154^+CD3^+$ T-cells in CMV IgG negative patients as compared to CMV IgG positive. The BTLA-4 expression was significantly decreased on $CD154^+CD3^+$ T-cells in CMV IgG negative patients as compared to CMV IgG positive.
Conclusion SLE-patients show a significant decreased expression of BTLA on CMV-specific T-cells. The co-inhibitors PD-1 and BTLA usually promote T-cell suppression. Thus a decrease may prone to severe symptomatic infections.

P96 THE REGULATION AND PHARMACOLOGICAL MODULATION OF IMMUNE COMPLEX INDUCED PRODUCTION OF TYPE III IFN BY PLASMACYTOID DENDRITIC CELLS

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Background Acknowledging the importance of type I interferon (IFN) in Systemic Lupus Erythematosus (SLE), we asked if RNA containing immune complexes (RNA-IC), which trigger the IFN-α synthesis by plasmacytoid dendritic cells (pDCs), also activate type III IFN (IFN-λ1-3) production, and how this is regulated.
Methods Peripheral blood mononuclear cells (PBMCs) were isolated from SLE patients and healthy individuals and depleted of monocytes. Immune cells were isolated from healthy PBMCs. Cells were stimulated with RNA-IC. Cytokines were measured by immunoassays, a microarray of pDCs, NK and B cells, as well as single-cell RNA-sequencing of pDCs was performed.
Results Type III IFN mRNA was induced in RNA-IC stimulated pDC-NK and pDC-B cell co-cultures, type III IFN was produced in pDC and pDC-NK cell co-culture supernatants. A small subset (3%) of RNA-IC activated pDCs expressed both IFNs type III and type I. Priming with IFN-λ2, IFN-α2b, interleukin (IL)-3, IL-6 and granulocyte-macrophage colony

stimulating factor (GM-CSF) significantly enhanced IFN-λ1/3 production by 2–5 fold. In pDC-NK cell co-cultures from SLE patients, IFN-α2b and GM-CSF increased the proportion of RNA-IC responding IFN-λ1/3 producing individuals from 9% to 36%. Hydroxychloroquine as well as an interleukin receptor 1 associated kinase 4 inhibitor (IRAK4i) significantly inhibited the RNA-IC-triggered IFN-λ1/3 production by pDCs and pDC-NK cell co-cultures by >90%.

Conclusions Type III IFN production in a small subset of pDCs can be triggered by RNA containing IC, enhanced by NK cells and several pro-inflammatory cytokines, and inhibited by blocking the TLR-MyD88 pathway, resembling the regulation of type I IFN. Thus, our results support a contributing role for both type I and type III IFN in SLE, which needs to be considered when targeting the IFN system in this disease.

P97 DEFICIENCY OF MARGINAL-ZONE B CELLS IN PERIPHERAL BLOOD OF SLE PATIENTS IN CLINICAL REMISSION OR LOW DISEASE ACTIVITY STATE IN A LONG-TERM STUDY

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Purpose Deficiency of marginal-zone B cells was observed in peripheral blood (PB) of SLE pts in clinical remission or low disease activity (LDA)[1]. Goal of the prospective, comparative, long-term study is follow-up of this phenomenon.

Methods Forty five adult SLE (ACR/1982, updated 1997) pts in complete remission or LDA[2] and 10 age- and sex-matched healthy controls (HC) were enrolled in „month 0', and SLE also after twelve-months („month 12') and 36-months („ month 36') period; overlap syndromes, infection, renal failure and monoclonal gammopathy in SLE were excluded. The DuraClone IM panel (Beckman Coulter) was used to identify $CD19^+CD27^+IgM^+$ B cell subpopulation in PB samples by flow cytometry navios (Beckman Coulter) with software analysis using Kaluza version 1.2.; data obtained were expressed in relative% of PB lymphocytes and absolute values $x10^6$/L, and processed using Medcalc-Statistical Software programme.

Results Significant differences ($p = 0.002$ - <0.001) were obtained between absolute values of $CD19^+CD27^+IgM^+$ B cells in HC (median 31.36, 95% CI 21.49 – 63.35) and SLE „month 0' (median 13.17, 95% CI 7.87 – 17.09), SLE „ month 12' (median 10.56, 95% CI 7.24 – 16.04), and SLE „ month 36' (median 9.66, 95% CI 7.22 – 13.21), but not between values obtained in SLE „month 0', „month 12' and „month 36' ($p>0.05$); not significant differences were found using analysis according to relative% of B cells under study ($p>0.05$).

Conclusion Data obtained demonstrated a long-term deficiency of marginal-zone B cells in PB of SLE pts in complete remission or LDA; susceptibility to infection should be supposed, but further studies are necessary.

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P98 NEUTROPHILS IN LUPUS: A NEW PHENOTYPE

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Background Polymorphonuclear neutrophils (PMNs) with irregular properties have been reported in patients with lupus and compelling evidence implicates them as a source of self-antigens. PMNs are the most abundant circulating leukocytes in human blood; defining their implication either as inherently defective players or as cells affected by external factors (e.g. autoantibodies, immune complexes and type I interferon) is of interest. We propose that elements present in the blood of patients with lupus affect neutrophil function/viability, in a NETosis-independent fashion.

Methods PMNs were isolated from venous blood of healthy volunteers and incubated with serum from normal subjects or from patients with lupus. Apoptosis and necrosis were assessed in real-time by cell surface exposure of phosphatidylserine (PS) and loss of plasma membrane integrity, respectively. Serums' analytes measurements were made by Luminex and ELISA.

Results Serums from patients with lupus caused a transient increase in PS exposure on the outer leaflet of PMN plasma membrane, in a significantly more intense fashion than serums from normal individuals. This peculiar phenomenon, which does not have characteristics of classic apoptosis, correlated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), in contrast to any of the following serum analytes: cytokines, chemokines, circulating growth factors, HMGB1, S100A8 and A9 proteins, complement components C3 and C4, immunoglobulins (IgA, IgG, IgM) and leukocyte counts. However, the transient PS increase was abolished by the decomplementation of the serums or by blocking the Fc receptors on PMNs' surface.

Conclusions Healthy PMNs are affected by the serum of patients with lupus. The transient PS exposure on the outer leaflet of the cellular membrane constitutes a new phenotype directly linked with factors that are at play in the blood of patients. Ongoing studies are looking at the nature of this new phenotype and how it links with PMNs being a potential source of self-antigens and/or as players unduly activated in lupus.

P99 EFFECTOR DN2 B CELLS ARE EXPANDED IN A MIXED ANCESTRY COLOMBIAN SLE PATIENT POPULATION

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Background Double Negative (DN) B lymphocytes are expanded in Systemic Lupus Erythematosus (SLE). Specifically, DN2 cells are a DN subset recently characterized and are functionally plasmablasts precursors. DN2 frequency is higher in SLE patients of African American ancestry and is associated