of spleen burden after i.p. infection (p<0.0001).
Vaccination with *B. suis* wt and Δ*mapB* OMVs induced systemic and mucosal specific humoral immune response, which may contribute to prevent *Brucella* mucosal entry and its dissemination.

171. (085) **TONSILLAR GERMINAL CENTER REACTIVITY REGULATION AND AGING**

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While tonsillar hyperplasia is the most frequent cause of tonsillectomy in children younger than 10, abscesses and acute infections are responsible for most tonsillectomies in teenagers. Wherever extracellular ATP (a DAMP) becomes abundant in the body, ectonucleotidases CD39 and CD73 are considered vital in the generation of an immunosuppressive microenvironment through adenosine production. Our goal was to investigate a potential metabolic adaptation of tonsillar B cells, promoting a suppressive behavior upon years of hyperplasia due to local chronic inflammation. By analyzing tonsillar mononuclear cells (TMC) using FACS, we compared co-expression of the ectonucleotidases CD73 and CD39 on CD20+ cells at different donor ages. We found that samples from older patients presented a statistically significant higher double positive CD73+ CD39+CD20+ cell population than those from younger children (37.7% ± SD 10% vs 25.8% ± SD 8.8% respectively, n=40, p<0.01). By culturing TMC with IL2/IL4/CpG/CD40L, we also show that activated B cells reliably downregulated CD73, presumably to prevent autocrine adenosine signaling. Thus, we found that neither IL10+CD20+ cells nor IL17+CD20+, generated upon stimulation, expressed CD73. In contrast, changes in CD39 expression with B cell activation resulted more variable between patients. Finally, we used the percentage of germinal center B cells (GC) as a read out of the effector immunological activity of the organs. We found that the proportion of GC within CD20+ cell population steadily declined with increasing age. GC B cells represented approximately one third of all the B cells from tonsils within the (2-9) year old range (29% ± SD 14%). That value declined to 15.7% ± SD 9.7% in tonsils from 10 to 18. Differences between the means were statistically significant (n=50, p<0.01). We concluded that the progression on the cause of tonsillar disease with age might illustrate the adaptation of the tonsillar tissue to constant inflammation.

172. (101) **B1 B CELLS ACQUIRE A PROLIFERATIVE AND ANTI-INFLAMMATORY PROFILE DURING PREGNANCY IN MICE**

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B1 B cells are a distinct subpopulation of B cells characterized by their unique capacity of self-renewal and the ability to secrete IgM without foreign antigen exposure (natural antibodies). In addition, upon activation, B1 B cells produce large quantities of the potent anti-inflammatory cytokine IL-10. Though the mechanisms that control natural antibodies production are not fully elucidated, it was recently associated with a down-regulation of CD1d expression in B1 B cells. Taking into account that both, IL-10 and natural antibodies are known to be fundamental components in pregnancy wellbeing, the aim of this study was to evaluate proliferation status as well as CD1d expression and IL-10 production by B1 B cells during pregnancy. Flow cytometry analysis, on splenic B1 B cells from pregnant (P) and non-pregnant (NP) mice was performed to evaluate ki-67 (proliferation marker) and CD1d expression as well as IL-10 production upon LPS stimulation.
We observed significantly higher expression levels of Ki-67 in splenic B1 B cells from P compared to NP (Unpaired t-test p<0,0001; n=3) mice which was mirrored by higher percentages of B1 B cells in the spleen of P mice (Unpaired t-test p=0,0095; n=11). In addition, B1 B cells from P mice expressed lower levels of CD1d as compared to NP mice (Unpaired t-test p<0,0001; n=3). Furthermore, LPS-stimulated B1 B cells from P mice produced significantly higher levels of IL-10 compared to NP mice *in vitro* (Unpaired t-test p=0,015; n=5).
Overall, our results demonstrate that not only B1 B cells are expanded in the spleen during pregnancy but they also seem to acquire the capacity to produce higher levels of natural antibodies and IL-10 during this period, suggesting their critical role in the intricate process of pregnancy tolerance.

173. (205) **EXTRACELLULAR ATP DRIVES T CELL IMBALANCE IN PEDIATRIC COVID-19**

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Background: Profound Tregs perturbations correlate with COVID-19 severity in adults. Extracellular ATP increases in inflammatory milieus with its concentration being regulated by CD39, mainly expressed on Tregs. There is no data regarding Tregs and Th17 cell balance in children with COVID-19.
Aims: 1) to analyze the frequency and phenotype of Tregs children with COVID-19 and controls; 2) to quantified the levels of ATP release by stimulated PBMCs and in serum; 3) to study the effect of extracellular ATP on CD4+ T cell balance.
Methods: We used sera, PBMCs and/or purified T cells from children with COVID-19 (n=54) and controls (n=24) to evaluate frequency and phenotype of cells subset and proliferative response by flow cytometry; ATP levels by luminometry; cytokines levels by multiplex assays.
Results: We observed a decreased frequency of Tregs in children with COVID-19, mainly in those with severe disease (p<0.01). These Tregs showed an activated phenotype with a strong suppressive profile including a great expression of CD25, CTLA-4 and CD39. Severe patients expressed increased levels of the ectonucleotidase cd39 in CD4+ T cells in comparison with non-severe (p<0.05) and controls (p<0.0001). Additionally, we found that stimulated PBMCs from severe children released the highest levels of ATP as well. We also detected that ATP promoted a fall in the proliferative response of purified T cells (p<0.0001) as well as in the Th1 and Th2 cytokine patterns. Interestingly, IL-17A and IL-17F levels did not decrease. As expected, ATP reduced the percentage of FOXP3+ and increased the expression of RORC in T cells, that were abrogated with the P2X7R antagonist, showing the involvement of this receptor. Finally, the levels of ATP in plasma correlated inversely with the frequency of Tregs.
Conclusions: We demonstrate that signaling through purinergic receptors drives Th17 but impairs Tregs immune response which have implications in the pathogenesis of pediatric COVID-19.

174. (229) **NOVEL HETEROZYGOUS MUTATION IN STX11 IN A PEDIATRIC PATIENT WITH EVANS SYNDROME**

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