density, light scattering and CD9 and CD63 contents. Unstimulated cells released primarily CD9+ EVs with buoyant densities of 1.13–1.19 g/ml, whereas IgE-mediated activation induced a massive and rapid release of CD9+CD63+ and CD63+CD9- EVs from MMC and CTMC, respectively. EV released by the activated CTMC were characterized by lower buoyant densities (1.21–1.23 g/ml). Functional analysis indicated that MC-derived EVs had a modulatory effect on dendritic cells and on cytokine production during antigen-driven $CD4^+$ T-cell priming. *Summary/conclusion*: The two major mast cell phenotypes MMC and CTMC release extracellular vesicles that differ in phenotypic characteristics after IgE-mediated activation and which show the ability to shape adaptive immune responses.

O-2C-4

Exosomes coated with antibody light chains bind antigen peptides on APC to antigen specifically suppress effector T-cells by delivery of miRNA-150

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Introduction: We previously described antigen (Ag)-specific suppressive exosomes derived from T & B cells in hapten-induced contact sensitivity (CS). We studied similar regulation of ovalbumin (OVA) protein-induced delayed-type hypersensitivity (DTH), focusing on the mechanism of suppression. In CS, the hapten-self peptide complexes on the Ag presenting cells (APC) cannot be analyzed. In contrast, OVA protein DTH allows analysis of the APC controlling the targeted effector T cells. *Methods*: According to Bryniarski et al. (J Allergy Clin Immunol 2013;132:170–181) inhibitory T-cell exosomes produced by CD8+ suppressor T (non-Treg) cells were induced by Ag high dose tolerance. They had a coating of B1a cell-derived Ag-specific antibody free light chains (Ab FLC) and a cargo of inhibitory miRNA-150. Suppressor B cell exosomes, that already expressed surface anti-OVA Ab FLC, were produced by B1a cells induced by intradermal Ag immunization and associated with miRNA-150. *Results*: Both suppressor T-cell exosomes containing miRNA-150 and B cell exosomes associated with miRNA-150 strongly inhibited DTH when injected systemically into mice actively sensitized with OVA. When the T or B cell exosomes were injected at the 24-hour peak of DTH, subsequent responses at 48–120 hours were inhibited by 60–80%. Importantly, and shown for the first time, orally administered T-cell exosomes caused even stronger inhibition, with 67–99% suppression of 48–120 hours DTH. To determine the nature of the Ag on the APC that Ab FLC coated exosomes binds, we studied 4 anti-OVA monoclonal Ab IgG antibodies that bound native OVA protein with markedly different strengths (strong, medium and weak) as did, but to a lesser extent, their FLC. Then we tested the suppressive ability of originally non-suppressive T-cell exosomes induced by Ag high dose tolerance in Ab deficient JH-/- mice. Separate groups of JH-/- tolerized exosomes were rendered Ag-specific by coating in vitro with FLC from each anti-OVA mAb. Resulting suppression of DTH via Ag-specific targeting likely of the Ag peptides of OVA on the APC surface was the inverse of their ability to bind native Ag. Two mAb FLC that hardly bound native OVA were the strongest mediators of exosome suppression. This suggested that the FLC coated exosomes may suppress by binding OVA peptides on the APC. *Summary/conclusion*: These results showed that Ag-specific T and B cell exosomes coated with Ab FLC can suppress OVA-induced DTH. Further, they were remarkably inhibitory orally. Interestingly, the mechanism of suppression seems to involve exosome-surface Ab FLC binding to Ag peptides complexed with MHC molecules on the targeted APC in an inverse manner to mAb ability to bind native OVA protein. Thus, AB FLC-coated exosomes carrying and delivering inhibitory miRNA-150 seem to bind OVA peptides on the APC surface and then subsequently to Ag to specifically suppress responses of their companion OVA/MHC-specific DTH-effector T cells.

O-2C-5

Defined breast milk EV subsets boost the immune response and skew the T-cell balance towards a regulatory phenotype

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Introduction: In the past years it has become clear that cell-derived extracellular vesicles (EV) are present in human breast milk and that these EV can play a role in the instruction of the immune system. Since breast milk impacts the development of the neonatal immune system by conveying environmental and maternal information to the child, we investigated the EV composition of human breast milk and evaluated the potential of these EV to modulate immune responses. *Methods*: For efficient and reliable recovery of naturally occurring EV from human breast milk, we applied a recently developed protocol, based on differential centrifugation and density gradient separation. Isolated EV were characterized by western blotting, EM, high-resolution flow cytometry, and lipidomics. For functional analysis of breast milk EV, removal of density gradient medium by column filtration was essential to avoid gradient medium-induced side-effects. EV-induced modulation of immune responses were analyzed in a T-cell stimulation assay with PBMC by profiling T-cell activation and cytokine release. *Results*: EV subsets were identified that differed in protein composition, light scattering, size and lipid composition. Furthermore, breast milk EV were also found to be highly enriched for several immune modulatory molecules, such as MHC class II, HSC/HSP-70, MFG-E8, butyrophilin 1A1 and MUC-1. Addition of breast milk EV to a T-cell stimulation assay revealed that milk EV skew T cells towards a regulatory phenotype, while boosting the release of the pro- and anti-inflammatory cytokines, such as IFN-g, TNF-a, IL-17, IL-10 and IL-6. *Summary/conclusion*: These data indicate that breast milk EV have a broad potential to steer immune responses, and could be transporters of (antigen-specific) immune information. As such, these EV may be involved in inducing immunogenicity, as well as oral tolerance in the neonate via the gastrointestinal tract.

O-2C-6

CD47 modulates T-cell micro-RNA expression and sorting into extracellular vesicles

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Introduction: Extracellular vesicles (EVs) mediate cell to cell communication in part by transferring mRNAs, miRNAs and other non-coding RNAs into recipient cells. The thrombospondin-1 receptor CD47 plays an important role in regulating communication between T cells and endothelial cells. EVs released by T cells alter gene expression and angiogenic signaling of recipient human umbilical vein endothelial cells (HUVEC) in a CD47-dependent manner (Matrix Biol 2014;37:49). Gene enrichment analysis of HUVEC treated with Jurkat T-cell-derived EVs also showed induction of T-cell signaling genes. *Methods*: To further examine the role of transferred RNAs, we performed global mRNA expression profiling of WT parental and CD47-deficient (JinB8) Jurkat T cells and their EVs and performed proteomic profiling of EVs. *Results*: Comparing Jurkat and JinB8 cells and EVs, 590 and 178 transcripts, respectively, were differentially expressed. EVs contained less mRNA than their cells of origin but