CD1 / NKT CELLS
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M.31. A Novel Pathogenic Mechanism of Recurrent Miscarriage Associated with β2glycoprotein I-dependent-antiphospholipid Antibody through CD1d on the Trophoblast

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Antiphospholipid antibody (aPL) is a risk factor for recurrent miscarriage (RM). aPL-related RM has been thought to result from intravascular coagulation in placenta. But recently β2glycoprotein (β2GPI) dependent aPL has been reported to harm trophoblasts directly and thereby cause RSA. CD1d is known to present self phosphatidylserine (PS) to invariant NKT cells. Here we addressed a novel pathogenic mechanism of RM associated with β2GPI-dependent aPL through the antibody crosslinking of CD1d. CD1d or CD1d/1a chimera genes were transduced to a human trophoblast Jeg cell and stable cell lines, Jeg3/CD1d or Jeg/CD1d/1a cells, were established. Flowcytometry revealed PS and β2GPI were expressed on the Jeg3/CD1d cell, but not Jeg cell. Interactions of PS and β2GPI with CD1d were confirmed by immunocytochemically and biochemically. CD1d Crosslinking by 51.1 mAb promoted IL-12 release from the Jeg/CD1d cell. Interestingly, crosslinking by anti-β2GPI mAb also promoted the IL-12 release for Jeg3/CD1d, but not Jeg3 or Jeg/CD1d/1a cells. We showed anti-β2GPI antibody interacts with CD1d on the trophoblasts through β2GPI-PS complex and induced CD1d crosslinking resulting in IL-12 release from trophoblasts. The IL-12 increase may activate decidual NKT or NK cells at maternal-fetal interface and thereby cause surplus inflammation in placenta and cause RM.

M.32. CD1d is Down-regulated in Human Papillomavirus (HPV)-associated Lesions through Proteasomal Degradation in a Manner Dependent on HPV E5 Protein

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CD1d plays a role in both innate and adaptive immune responses to microbes. Many microbes down-regulate CD1d for their immunoevasion. HPV E5 protein resident in ER is reported to involve down-regulation of MHC molecules. We here addressed CD1d alteration in HPV-infected cell. Immunoreactivity of cervical intraepithelial neoplasia (CIN), cervical cancer, or condyloma lesions for CD1d was assessed by immunostaining with NOR3.2 mAb. CD1d gene was transduced stably into a CD1d-negative and HPV-negative cervical cancer cell C33a. HPV6E5 or 16E5 genes were transduced into the CD1d-expressing C33a. Alterations in CD1d were assessed by RT-PCR for mRNA, western blotting and immunostaining for protein, or flow cytometry for surface expression. To see mechanism of CD1d alteration, the E5-expressing cells were treated with a proteasome inhibiter MG132. CD1d immunoreactivity decreased in high-grade CIN or condyloma and lost in cancer lesions. Although CD1d mRNA level did not alter, western blotting and immunostaining revealed CD1d protein was down-regulated in 6E5- or 16E5-expressing cells. Flow cytometry confirmed the decreased cell-surface CD1d in the E5-expressing cells. CD1d was rescued by MG132 treatment indicating CD1d decreased by proteasomal proteolysis. CD1d was down-regulated in HPV-infected cells by HPV E5-induced proteasomal degradation and this may result in immunoevasion of HPV.

M.33. Deficiency of Invariant Natural Killer T Cells Leads to Increased Inflammation and Tumorigenesis in Colitis-associated Colon Cancer Model

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Background and Aim: Invariant natural killer T (iNKT) cells are considered innate-like lymphocytes, and regulate the immunity against inflammation and tumorigenesis. In this study, we examined the physiologic role of iNKT cells in a mouse colitis-associated colorectal cancer model. Methods:C57BL/6 (B6) and Jα18 NKT cell-deficient KO (KO) mice were used. Colitis-associated colorectal cancer was induced by azoxymethane (AOM) and dextran sodium sulfate (DSS). The tumors and inflammations were examined. The surface markers of mononuclear T cells from the liver and the colon were assessed by FACS. Cytokine secretions in MLN were measured by ELISA. Results: In AOM/DSS model, hepatic iNKT cells were significantly decreased. KO mice showed significantly more numbers of colon tumors and severe inflammation than B6 mice. FACS revealed that there was no obvious change between B6 and KO mice in the frequencies of lamina propria NK cells, and CD8+T cells. However, the populations of NK1.1+NKT cells, CD25lowT cells, and CD25highT cells, in KO mice were increased as compared to B6 mice. There were no difference in the secretion of IFN-γand TNF-α. Conclusion: iNKT cells may play a critical role on the prevention from tumor progression and inflammation on the AOM/DSS model.

M.34. Impact of Nod Signaling on NKT Cell Effector Function

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Invariant natural killer T (iNKT) cells are a subset within NK T cells that express the Vα14-Jα8 TCR α chain. The signaling for iNKT cell activation is not clear but preliminary work from our laboratory suggests that ligands for the nucleotide-binding oligomerization domain (Nod) proteins may modify NKT function. Both iNKT cells and Nod proteins are known to skew toward a Th2 bias. Our hypothesis is that Nod proteins can

impact on iNKT cell function and thereby alter the balance of Th1 versus Th2 development. Since polymorphisms in the Nod1 gene have been linked to asthma, we propose that in the setting of this disease, dysfunctional Nod1 signaling in NKT cells might contribute to abnormal cytokine and antibody levels. Our preliminary findings suggest that iNKT cells can be induced to produce IFN-γ *in vitro* by DCs stimulated with Nod ligands. In parallel, *in vivo* studies priming wild-type mice with Nod ligands and analysis of MNCs from the liver also suggest Nod1 signaling might contribute to IFN-γ production by NKT cells. Studies are ongoing to examine antigen-specific antibody production in NKT cell-deficient mice primed with Nod agonists. Taken together, our preliminary findings demonstrate a role for Nod signaling in NKT function.

M.35. Regulation of Autoimmune Diabetes by CD1d-restricted Type 2 Natural Killer T Lymphocytes

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CD1d-restricted natural killer (NK) T lymphocytes are divided into two subgroups based on the T cell receptor (TCR) they carry. Type 1 NKT cells utilize an invariant Vα14Jα18 TCR α-chain. Type 2 NKT cells are CD1d-restricted T cells displaying other, diverse TCR. Recent reports suggest that type 1 and type 2 NKT cells display distinct functions, however the immunological role and functional attributes of type 2 NKT cells are less well studied. We have previously demonstrated that TCR-transgenic type 2 NKT cells suppressed type 1 diabetes (T1D) in the non obese diabetic (NOD) murine model for the human disease. To further elucidate the requirements for immuno regulation by type 2 NKT cells, we have used an accelerated disease model, in which T1D is induced by the transfer of diabetogenic TCR transgenic CD4+ BDC2.5 T cells into NOD.scid mice. Co-transfer of type 2 NKT cells with BDC2.5 CD4+ cells prevented disease. Protection required CD4+ NKT cells, but was independent of CD25+ T regulatory cells. NKT cells were found in lymphoid organs as well as pancreas of protected recipients. Experiments are under way to address the underlying mechanism of how type 2 NKT cells modulate T1D induction by BDC2.5 T cells.