

and those inducible into FVT and PVT/VF still had a risk of appropriate ICD therapy for spontaneous ventricular tachyarrhythmias of 5%/year, suggesting that the induction of MVT as the sole predictor of future arrhythmic events may be inadequate.

Cox Proportional Hazard Ratios

Variable	Hazard Ratio (HR)	95.0% Confidence Interval for HR		p Value
		Lower	Upper	
Noninducible vs PVT/VF	1.134	0.448	2.869	.791
FVT vs VF	0.588	0.209	1.651	.313
MVT vs VF	2.209	1.008	4.840	.048
Male sex	1.451	0.703	2.996	.314
Ischemic cardiomyopathy	0.629	0.363	1.090	.099
Ejection fraction %	0.977	0.960	0.994	.008
Primary vs secondary implant indication	1.906	0.662	1.814	.722

87

DENDRITIC CELL-DEPENDENT INDUCTION OF BETA CELL-SPECIFIC REGULATORY T CELLS FOR SUPPRESSION OF AUTOIMMUNE DIABETES. K. Pothoven, K. Tarbell, H. Yang, R.M. Steinman, M. Suthanthiran, X. Luo, Northwestern University, Chicago, IL; New York, NY.

Background: Thymic-derived CD25⁺CD4⁺ regulatory T (Treg) cells have been found to play an important role in the pathogenesis of autoimmune diabetes. Challenges for their application as a potent immunomodulatory therapy are (1) the small size of the naturally occurring CD25⁺CD4⁺ Treg population and (2) the polyclonal nature of the existing CD25⁺CD4⁺ Treg cells. Here we describe a novel system of using dendritic cell (DC)-stimulated expansion in the presence of TGF- β 1 for in vitro generation of beta cell-specific CD25⁺CD4⁺ T cells that are potent suppressors of autoimmune diabetes. **Material and Methods:** Naive BDC2.5/NOD CD25⁺CD4⁺ cells were obtained by cell sorting from pooled BDC2.5/NOD LNs. Splenic DCs from NOD mice were purified by CD11c-positive selection. Naive CD25⁺CD4⁺ T cells were either cultured with irradiated CD11c⁺ DCs and BDC peptide (specific stimulation) or with anti-CD3 and anti-CD28 (nonspecific stimulation) for 7 days with or without TGF- β 1, after which the CD25⁺ T-cell fraction was purified and analyzed. **Results:** Purity of the BDC2.5/NOD CD4⁺CD25⁺ was routinely > 97%. At baseline, the CD4⁺CD25⁺ BDC T cells express minimal Foxp3 measured by FACS analysis and real-time PCR. Stimulation in the presence of TGF- β 1 with either specific or nonspecific conditions leads to marked induction of Foxp3 expression to a level comparable to that seen in naturally occurring CD25⁺CD4⁺ Treg cells. This induction was not seen in the absence of TGF- β 1. The induced CD25⁺CD4⁺Foxp3⁺ BDC T cells generated with DCs plus BDC peptide (specific stimulation) maintained a high level of cell surface clonotype expression after stimulation and exert antigen-specific suppression in in vitro suppression assays. When cotransplanted with syngeneic islets in diabetic NOD mice, these cells significantly prolonged islet graft survival from a median of 12 to 46 days ($p = .0008$). When cotransferred with diabetogenic cells into NOD.scid recipients, these cells significantly delayed the kinetics of diabetes onset ($p < .0001$). In contrast, CD25⁺CD4⁺Foxp3⁺ BDC T cells induced with anti-CD3 and anti-CD28 (nonspecific stimulation) show lower levels of clonotype expression on cell surface and were unable to suppress BDC peptide-stimulated proliferation in vitro or protect islet grafts in vivo. **Conclusion:** Beta cell-specific BDC2.5 CD25⁺CD4⁺ cells with high levels of Foxp3 can be induced from naive BDC2.5 CD4⁺CD25⁺ cells by TGF- β 1 in CD11c⁺ DC-stimulated expansions. These cells harbor potent suppressive activity in an islet antigen-specific manner and suppress autoimmune diabetes.

88

EFFECT OF THE S1P1 GENE KO ON LIPOPOLYSACCHARIDE-INDUCED MURINE ACUTE LUNG INJURY. S. Sammani, S. Mirzapoiazova, L. Moreno, R. Proia, C. Ewenoski, J. Moitra, V. Natarajan, P. Singleton, J.G. Garcia, University of Chicago, Chicago, IL; Bethesda, MD.

Acute lung injury ALI/ARDS, a significant cause of morbidity and mortality, is characterized by a diffuse inflammatory parenchymal process with pulmonary EC vascular leak and alveolar flooding. This syndrome remains a significant cause of intensive care unit mortality, and more effective therapeutic interventions are needed. Our in vitro studies indicate that sphingosine 1-phosphate (S1P), a phospholipid angiogenic factor and a major barrier-protective product of platelets, produces endothelial cell barrier enhancement through ligation of the S1P family of receptors, especially S1P1, a G protein-coupled receptor expressed on vascular endothelial cells. Our previous data show that S1P, via S1P1, has impressive protective effects in both murine and canine models of ALI (McVerry et al, 2004). To better understand S1P receptors in barrier regulation, we examined LPS-induced ALI in S1P1 receptor heterozygous (S1P1R^{+/−}) mice. Our data demonstrate that the S1P1-R^{+/−} mice exhibit increased barrier disruption compared with wild-type mice, reflected by an increase in protein (25%) and inflammatory cell count (20%) in bronchoalveolar lavage (BAL) fluid. To confirm the role of S1P1R on the S1P barrier-protective effect, we administered S1P (UM final blood concentration, iv) simultaneously with LPS (2.5 mg/kg) and evaluated lung inflammation 18 hours later. LPS-treated wild-type mice treated with S1P demonstrated profound reductions (> 50%) in BAL protein, whereas S1P1^{+/−} mice similarly treated with S1P only exhibited only a \approx 10% increase in BAL protein. In conclusion, our data using genetically engineered mice demonstrate a critical need for S1P1 receptors in vivo, particularly in conditions of endotoxemia.

89

LIPID RAFT REGULATION OF HEPATOCYTE GROWTH FACTOR/C-MET-MEDIATED VASCULAR INTEGRITY: ROLE OF CD44, TIAM1/RAC1, DYNAMIN 2, AND CORTACTIN. P.A. Singletar, R. Salgia, L. Moreno-Vinasco, J. Moitra, S. Sammani, T. Mirzapoiazova, S.M. Dudek, J. Garcia, University of Chicago, Chicago, IL.

Endothelial cell (EC) barrier dysfunction results in increased vascular permeability, a feature of inflammation, tumor angiogenesis, atherosclerosis, and acute lung injury. Therefore, agents that protect

vascular integrity have important therapeutic implications both in vivo and in vitro. We have previously shown that the binding of hepatocyte growth factor (HGF) to its cell surface receptor, c-Met, promotes Rac1-dependent increases in lung EC barrier function. Further examination of the regulatory mechanisms of HGF/c-Met-induced EC barrier enhancement revealed that HGF (25 ng/mL) promotes c-Met recruitment into specialized caveolin 1-enriched plasma membrane microdomains (lipid rafts). Abolishing lipid raft formation (M β CD) attenuated HGF-induced EC barrier enhancement. Within lipid rafts, HGF induced c-Met association with CD44 (a major glycoprotein receptor for hyaluronan). Silencing CD44 expression (siRNA) inhibited HGF-induced c-Met and Tiam1 (a Rac1 guanine nucleotide exchange factor) recruitment to lipid rafts, as well as the association of cortactin (an actin-binding regulatory protein) with dynamin 2 (a vesicular regulatory protein) within lipid raft structures. Silencing of either Tiam1 or dynamin 2 blocked HGF-induced Rac1 activation, cortactin recruitment to lipid rafts, and EC barrier enhancement. Silencing cortactin attenuated HGF-induced EC barrier regulation. Finally, HGF-mediated in vivo protection from lipopolysaccharide-induced pulmonary vascular hyperpermeability was inhibited in CD44 knockout mice. Taken together, these results suggest that lipid rafts are an essential regulator of HGF/c-Met-mediated barrier enhancement via a process involving CD44, Tiam1, Rac1, dynamin 2, and cortactin.

90

GASTRIC CANCER PRESENTING AS SUBACUTE COMBINED DEGENERATION OF THE SPINAL CORD. P. Sircar, S. Shetty, B. Cheeran, E. Akinymi, S. Niranjan, Coney Island Hospital, Brooklyn, NY.

Introduction: In clinical practice, it is not uncommon to come across cases of subacute combined degeneration of the spinal cord in patients with pernicious anemia. The commonest cause of cobalamin deficiency is due to inadequate absorption associated with pernicious anemia. Vitamin B₁₂ deficiency is also associated with gastrectomy and autoimmune metaplastic atrophic gastritis. Although chronic atrophic gastritis can lead to an increased risk of intestinal-type gastric cancer and gastric carcinoid tumor, presumably owing to prolonged achlorhydria resulting from parietal cell loss, it is very unusual for neurologic complications to be the primary manifestation in a patient with gastric cancer. We present a rare case of gastric adenocarcinoma presenting initially with neurologic complications of B₁₂ deficiency. **Case Report:** A 48-year-old Caucasian gentleman came to the hospital complaining of gait disturbance for 3 to 4 weeks. Symptoms began with paresthesia in the lower extremities and gradually progressed to a point where he was unable to walk without support. He also complained of early satiety and dyspepsia for a few months. His past medical history and family history were essentially unremarkable. He drank moderate amounts of alcohol on a regular basis and also smoked one pack of cigarettes a day, both for 20 years. On examination, his abdomen was benign, but he had significant neurologic findings. His mental status and cranial nerves were intact. Motor strength was 4/5 in both lower extremities and tone was increased bilaterally. Sensory examination revealed hypoesthesia in both lower extremities. Vibration and position sense were absent bilaterally. Plantars were equivocal. Initial laboratory data were consistent with macrocytic anemia (peripheral smear showing hypersegmented neutrophils), with hemoglobin of 9 g/dL and MCV of 110 fL. The vitamin B₁₂ level was on the lower side (210 pg/mL), with an increased B₁₂ binding capacity (1,637 pg/mL). Radiologic investigations of the spine were unremarkable, including MRI of the spine. The patient also underwent upper gastrointestinal endoscopy for evaluation of his dyspeptic symptoms, which revealed poorly differentiated gastric adenocarcinoma, well to moderately differentiated, in the background of acute and chronic gastritis. The specimen was negative for *Helicobacter pylori*. The patient was treated with vitamin B₁₂, and his neurologic symptoms improved dramatically. He also underwent partial gastrectomy and did well thereafter. **Discussion:** The lesion in subacute combined degeneration of spinal cord is specific for cobalamin deficiency and is proposed to be due to a defect in myelin formation of unknown mechanism. Symptoms begin with paresthesia and ataxia associated with loss of vibration and position sense and can progress to severe weakness, spasticity, clonus, paraplegia, and even fecal and urinary incontinence. Early treatment with vitamin B₁₂ can prevent many complications and permanent disability. Patients with atrophic gastritis may have an increased risk of developing gastric or colorectal adenocarcinoma, but the data are not entirely conclusive. There are no set recommendations to screen these patients at a greater frequency than the general population. Nevertheless, it is prudent to periodically monitor stools in these patients for the presence of blood. As the above case represents, gastric carcinoma rarely have an unusual presentation like our patient had, and a high index of suspicion for it can potentially be lifesaving in an otherwise near-fatal diagnosis.

91

METASTATIC MALIGNANT MELANOMA PRESENTING AS MASSIVE LOWER GASTROINTESTINAL BLEEDING. P. Sircar, D. Godkar, J. Balachandran, S. Niranjan, Coney Island Hospital, Brooklyn, NY.

Introduction: Malignant melanoma represents 1 to 3% of cancers in the United States, and its prevalence is steadily increasing. Metastatic melanoma of the small bowel is a pathologic entity that is infrequently reported, and antemortem diagnosis is made in only 1.5 to 4.4% of all patients with melanoma. Gastrointestinal metastases may manifest as mucosal or submucosal masses, serosal implants, or carcinomatosis. They arise more commonly in the mesentery or distal small bowel than the proximal gastrointestinal tract or colon. Rarely are these lesions symptomatic; sometimes patients present with pain, obstruction, and occult or overt gastrointestinal bleeding. We present an unusual case of metastatic malignant melanoma of the second part of the duodenum presenting as life-threatening gastrointestinal hemorrhage. **Case Report:** A 53-year-old Russian lady came to the emergency room after an episode of syncope. She also complained of progressively worsening shortness of breath and fatigue for a few months. Her past medical history was remarkable for a history of malignant melanoma of the skin diagnosed 3 years ago, for which she had received excisional surgery and had remained free of recurrence for 3 years. Physical examination was significant for extreme pallor and multiple pigmented skin lesions of variable sizes on the trunk and back. She was neurologically intact, and the abdomen was benign, but rectal examination revealed soft dark stool, strongly guaiac positive, without masses or lesions. Initial laboratory work revealed a hemoglobin of 5.7g/dL, with microcytic red cell indices (MCV 62.1 fL). Her electrolytes, liver function tests, and renal function tests were all within normal limits. Chest radiography revealed multiple parenchymal lesions bilaterally, and CT of the brain with intravenous contrast showed multiple lesions in the brain parenchyma without mass effect. The patient was transfused two units of packed red cells and underwent endoscopic studies to evaluate the cause of her profound anemia and was found to have a mass lesion at the second part of the duodenum. She underwent duodenoscopy with biopsy of the lesion, which revealed metastatic malignant melanoma (immunohistochemical stains showing S-100 and melan-A positive). Also, biopsy from the skin lesions at the back revealed malignant melanoma arising from compound nevus, with level III invasion, and a tumor thickness of 0.8 mm. The patient did not bleed any further from her