background (NOD-S2+/-) to test the role of ER Ca2+ loss during T1D development. Senescence associated βgalactosidase staining (SA-βgal), expression of senescence markers (RT-qPCR), mitochondrial function (Seahorse, TMRM) and mitochondrial copy number (qPCR) were all measured in S2KO versus WT βcells and are currently being measured in the NOD-S2+/- mouse model at 6, 8, 12, 14, and 16wks of age. RESULTS/ANTICIPATED RESULTS: RT-qPCR assays detecting senescence markers cdkn1a and cdkn2a and mitochondrial specific genes cox1 and nd1 were developed and validated in both INS-1 βcells and mouse islets. Mitochondrial function assay (Seahorse) was optimized for use in INS-1 βcells and is currently under development for use in intact mouse islets. S2KO βcells displayed increased SA- βgal staining as well as increased mitochondrial coupling efficiency (p=0.0146) and baseline mitochondrial copy number (p=0.0053) compared to WT βcells, suggesting a senescence phenotype and altered mitochondrial function. NOD-S2+/- mice exhibited increased expression of the senescence marker cdkn2a in the islet at 12wks (p=0.0117) compared to control mice, whereas cdkn1a remained unchanged across all timepoints tested. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our results suggest that loss of SERCA2 and reduced ER Ca2+ alter βcell mitochondrial function and are associated with features of senescence. Future studies will test whether SERCA2 activation and/or senolytic/senomorphic drugs are able to prevent or delay diabetes onset in NOD-S2+/- mice.

70759

Jaw-specific control of Msx1-dependent odontogenesis by Dkk2 and Sostdc1

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ABSTRACT IMPACT: Our proposed jaw-specific control mechanism of tooth development is expected to address the site-specific prevalence of tooth agenesis in humans. OBJECTIVES/GOALS: To determine the molecular mechanisms that control jaw-specific tooth development. To identify the molecular basis of the site-specific prevalence of humans tooth agenesis cases. METHODS/STUDY POPULATION: We used three different genetically engineered mouse lines: ***Msx1 ^'/ ^', Dkk2 ^'/ ^', and Sostdc1 ^'/ ^' mice. We used developmental mouse genetics approaches, basically generating different combinations of compound mutant mice. We examined their tooth development by using gross, histology, and mRNA expression analyses. RESULTS/ANTICIPATED RESULTS: We identified that Sostdc1, a secreted Wnt inhibitor, also plays an important role in regulating the Msx1-dependent odontogenic pathway. Sostdc1 mRNA showed similar expression patterns in the developing tooth germs between control and Msx1-null molar buds. Remarkably, by deleting the Sostdc1 gene, as well as the Dkk2 gene, in the Msx1-null background mouse, molar tooth development was rescued in the maxillary jaw, but not in the mandibular jaw. Furthermore, tooth developmental rescue could be achieved in both the maxillary and mandibular molars by combinedly deleting Dkk2 and Sostdc1 in Msx1-null mice. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our study demonstrates that secreted Wnt inhibitors Dkk2 and Sostdc1 synergistically regulate the Msx1-dependent odontogenic pathway and further control early tooth morphogenesis. These mouse model will be used to further address the site-specific prevalence of tooth agenesis in humans.

72399

Epigenetic Modification of Macrophages Contribute to Protective Memory in Against Staphylococcus aureus

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ABSTRACT IMPACT: This work may provide new targets for vaccine and immunotherapeutic development against MRSA infections. OBJECTIVES/GOALS: Staphylococcus aureus is the leading cause of skin and skin structure infection (SSSI), a primary portal of entry for invasive infection. Patients with SA SSSI have a high 1-year recurrence. We have shown innate memory protects mice against SA SSSI. The goal of this project is to determine epigenetic mechanisms of protective memory against SA SSSI. METHODS/STUDY POPULATION: We have shown macrophages (Mf) afford protective memory against recurrent SA SSSI in mice. Priming by prior infection reduced skin lesion size and MRSA burden, which correlated with increased Mf in abscesses and lymph nodes. Priming potentiated the opsonophagocytic killing of SA by bone-marrow derived Mf (BMDM) in vitro, and their adoptive transfer into naive skin afforded protective efficacy in vivo. Here, we investigated epigenetic mechanisms of anti-SA efficacy in BMDMs. BMDM from naive (uninfected) or primed (SA SSSI) wild-type C57Bl/6 mice were cultured ex vivo. DNA from BMDM groups were isolated and analyzed for methylation changes using reduced representation bisulfite sequencing (RRBS). Pathway analyses of methylation changes were determined with Panther. RESULTS/ANTICIPATED RESULTS: Present findings indicate the protective memory afforded by BMDM was mediated by epigenetic modifications of the DNA. Using RRBS, we profiled differentially methylated regions (DMR) in DNA from naive vs. primed BMDM. Primed BMDM exhibited significantly different DMRs as compared to naive BMDM. Proximity to known genes were mapped using GREAT. Pathway analyses revealed DMRs predominant in genes integral to immune modulation, such as integrin signaling, cytokine/chemokine networks, and growth regulation. For example, SA-primed BMDM were hypermethylated proximate to GIMAP8 versus naive BMDM, suggesting repression of this protein. Gimap family ligands are small GTPase immune-associated proteins expressed in immune cells known to regulate macrophage lysosomal fusion during parasite infection. DISCUSSION/SIGNIFICANCE OF FINDINGS: These findings reveal epigenetic mechanisms of macrophage innate memory against recurrent MRSA infection. Functional testing of these genes in response to SA infection is needed to confirm their protective role. These insights may provide new targets for vaccine and immunotherapeutic development against MRSA.

79664

Complement Driven Auto-Reactive Antibodies in Lung Transplantation*

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ABSTRACT IMPACT: Our work unveils a novel mechanism of ischemia repurfusion injury driven by pre-existing autoimmunity following lung transplant and a potential therapeutic strategy for

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blocking complement-dependent injury thereby reducing risk of lung transplant rejection. OBJECTIVES/GOALS: Our goal was to determine if pre-existing autoimmune autoantibodies, such as those resulting from cigarette smoke (CS), contribute to graft rejection in lung transplantation (LTx) and if autoreactive-mediated graft injury is complement-dependent. METHODS/STUDY POPULATION: For in vivo experiments, we utilized our emphysema mouse model. Briefly, eight-week-old C57BL/6J mice are exposed to 3R4F reference cigarette smoke 5 hours per day, 5 days a week for 6 months. Upon completion, cigarette smoked (CS) mice and control (NS) mice received syngeneic orthotopic left-lung transplant from age-matched C57BL/6J donors. To determine if pre-existing autoreactivity mediated graft injury was complement-dependent we treated CS-LTx mice with a novel, bifunctional complement inhibitor. Autoantibody levels were measured by ELISA and lung injury was assessed by blinded histopathological analyses. Complement inhibition was verified by immunofluorescence. RESULTS/ANTICIPATED RESULTS: We found that CS-exposure leads to production of autoreactive antibodies towards extracellular matrix (ECM) components and contributes to graft injury. Interestingly, LTx into CS exposed mice further increased de-novo ECM autoantibody development. Lastly, treatment with our novel, bifunctional complement inhibitor blocked autoantibody spreading and significantly reduced graft rejection. DISCUSSION/SIGNIFICANCE OF FINDINGS: These data demonstrate that smoking induces pre-LTx autoreactivity to ECM proteins that promotes graft injury following LTx. Furthermore, complement inhibition reduces autoantibody production and protects the graft from injury.

89723

CD105-targeted CAR T cells for the treatment of acute myeloid leukemia*

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ABSTRACT IMPACT: Our work might lead to a new treatment for patients with acute myeloid leukemia OBJECTIVES/GOALS: Acute myeloid leukemia (AML) is a devastating hematologic malignancy, with dismal 5-year survival. Chimeric antigen receptor (CAR) T cells have been approved for B cell malignancies but not for AML. The goal of this study is to explore the safety and efficacy of CAR T cells targeting CD105 (endoglin) to treat AML. METHODS/STUDY POPULATION: We have constructed human and murine CAR T cells targeting CD105. The CARs were created by sequencing the V(D)J regions of hybridomas and designing single chain variable fragments that target CD105 which were subsequently introduced in a CAR backbone via Gibson assembly. The CAR T cells were produced via transduction using retrovirus or lentivirus. Leukemia cell lines were assessed for CD105 expression with flow cytometry. Killing assays were performed via measurement of luminescence of target cells after co-culture with CAR T cells. Activation assays were performed with co-culture of CAR T cells and target cells and measurement of activation markers with flow cytometry. To assess in vivo efficacy and safety, murine CAR T cells were infused into C57BL/6J mice carrying B16 melanoma after lymphodepletion. RESULTS/ANTICIPATED RESULTS: All human leukemia cell lines assessed (Nalm6, MOLM-14, MV4-11, Kasumi-1, THP-1) expressed some degree of endoglin apart from the T cell leukemia Jurkat. Human CD105 CAR T cells were activated by co-culture with leukemia cell lines and effectively killed leukemia cells in vitro in a CD105-specific manner. Murine CAR T cells killed efficiently both murine solid tumors (B16 melanoma) and murine leukemias (C1498) in vitro. Murine CAR T cells did not exhibit any toxicity when infused after low-dose lymphodepletion (cyclophosphamide 100mg/kg) but caused significant morbidity after higher doses (cyclophosphamide 200mg/kg). Murine CAR T cells delayed the growth of B16 melanoma in immunocompetent mice. DISCUSSION/SIGNIFICANCE OF FINDINGS: We have constructed human and murine CD105 CAR T cells with excellent activity in vitro. The activity of human CD105 CAR T cells in xenografts and the biologic relevance of the toxicity of murine CD105 CAR T cells in humans needs to be further investigated. CD105 CAR T cells might prove an important therapeutic option for patients with AML.

91074

Identification of monoclonal antibodies with broad reactivity against the malaria parasite variant surface antigen responsible for severe malaria†

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ABSTRACT IMPACT: This study aims to provide insight into naturally acquired immunity against severe malaria, thereby laying the foundation for the design of novel vaccine candidates to prevent severe disease as well as monoclonal antibody therapies to treat severe malaria. OBJECTIVES/GOALS: Severe malaria is caused by parasite surface antigens that contain high sequence diversity. Nevertheless, P. falciparum-exposed individuals develop antibody responses against these antigens. Our goal is to isolate antibodies with broad reactivity to understand how disease protection is acquired. METHODS/STUDY POPULATION: Our study cohort consists of Ugandan adults living in a malaria-endemic region with high transmission intensity, who are protected against severe malaria. Using fluorescently labeled probes of parasite surface antigens, we have isolated antigen-specific B cells from these donors. We then expressed the corresponding monoclonal antibodies in vitro. These antibodies were screened against a library of variant surface antigens to determine antibody breadth and potential to inhibit interaction of the parasite surface antigen with host receptors, a critical step in pathogenesis. Additionally, using a panel of variant surface antigen mutants, we have predicted the epitopes targeted by the broadest monoclonal antibodies. RESULTS/ANTICIPATED RESULTS: We have identified three monoclonal antibodies with exceptionally broad reactivity and inhibitory activity against our panel of severe disease-inducing variant surface antigens. We have identified two major sites targeted by these broadly reactive antibodies. The first site was associated with the largest breadth, but limited inhibitory potential, while the second site showed high-affinity antibody binding and inhibition of receptor binding. Interestingly, two of these three antibodies were very similar in structure, even though they were isolated from different donors. Isolation of antigen-specific B cells from additional donors will enable us to identify how common such broadly reactive antibodies are and allow the identification of additional epitopes DISCUSSION/SIGNIFICANCE OF

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