biologic therapy directed against TNFα (anti-TNFα, Infliximab) and α4β7 integrin (anti-α4β7; Vedolizumab) is used to treat IBD, a substantial number of patients remain non-responsive. Using a comprehensive bioinformatics approach, the aim of this study was to characterize immune cell profiles and altered molecular pathways in IBD patient non-responders to anti-TNFα and anti-α4β7 therapy to determine potential mechanisms and/or indicators of treatment non-response. METHODS/STUDY POPULATION: Publicly available whole transcriptomes from 65 healthy control and IBD endoscopic biopsies were assessed (NCBI GEO GSE73661). Specifically, transcript profiles from responders or non-responders to anti-TNFα and anti-α4β7 therapy were utilized. Differentially expressed transcript profiles were obtained by comparing responders or non-responders prior to receiving therapy versus healthy controls using NCBI's GEO2R after adjustment with Benjamini and Hochberg testing ($p<0.05$). Immune profiling of DEgs were analyzed by the core LM22 immune signature for subsets of B-, T-, dendritic-, mast-cells, macrophages, and neutrophils (CIBERSORT, cibersort.stanford.edu) ($p<0.05$). Networks, functional analysis, and interpretation of transcriptomic data were performed using Ingenuity Pathway Analysis (IPA) (Qiagen) ($p<0.05$). RESULTS/ANTICIPATED RESULTS: Initially, we determined colonic immune profiles in responders and non-responders to anti-TNFα and anti-α4β7 therapy. Compared to responders, in both anti-TNFα and anti-α4β7 non-responders we found elevated neutrophil levels ($p<0.05$). Specific to anti-TNFα treatment, non-responders demonstrated substantially reduced Treg cells ($p<0.05$); whereas, exclusive to anti-α4β7 treatment, non-responders showed elevated dendritic cells, activated CD4 T cells, and reduced M2 macrophages ($p<0.05$). Next we profiled differentially expressed transcripts to determine molecular pathways associated with therapy non-response. In both anti-TNFα and anti-α4β7 non-responders, we observed alterations in pathways specific to cellular growth and metabolism. Among cell growth pathways we found activated growth hormone, Wnt, ErB, and IGF-1 signaling; whereas, among metabolic regulation we found altered triglyceride, tryptophan, and leptin signaling. Moreover, unique to anti-TNFα non-responders, we found activated sphinogosine-1-phosphate and paxillin pathways. While non-response to anti-α4β7 indicated activation of SAPK/JNK and IL-9 signaling. DISCUSSION/SIGNIFICANCE OF IMPACT: Together these data define specific immune profiles and molecular pathways observed in non-responders to anti-TNFα and anti-α4β7 therapy. Our analysis identified substantial alterations in pathways specific to cellular growth and metabolism, identifying a link between non-response to biologic therapy and specific cell functions. These data suggest particular alterations in immune profiles and molecular pathways could play a role in non-response to biologic therapy, highlighting a future direction for personalized treatment regimens that could lead to more targeted use of existing therapies and more favorable patient health outcomes.

3005

Integrin Mac-1 Potentiates Neutrophil Adhesion and NET Release in Antiphospholipid Syndrome

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OBJECTIVES/SPECIFIC AIMS: While the role of antiphospholipid antibodies in activating endothelial cells has been extensively studied, the impact of these antibodies on the adhesive potential of leukocytes has received considerably less attention. Mac-1 is a heterodimeric beta-2 integrin primarily expressed by myeloid-lineage cells. In its activated state, Mac-1 mediates cell-cell interactions by engaging a variety of surface molecules, including the endothelium-expressed glycoprotein ICAM-1. Here, our goals were (1) to determine the extent to which APS neutrophils adhere to healthy, resting endothelial cells under physiologic flow conditions, and (2) to identify potential therapeutic targets by elucidating the molecules required for that adhesion. METHODS/STUDY POPULATION: Primary APS patients (meeting Sydney criteria) and non-autoimmune controls were matched for age and gender. Freshly isolated human umbilical vein endothelial cells (HUVECs) were utilized within five passages. Samples were introduced into a flow channel via a programmable syringe pump, and perfused across a resting HUVEC monolayer. After 15 minutes of perfusion, the chamber was flushed, and the remaining adherent cells were quantified. Flow cytometry was used to identify differentially-expressed molecules on the surface of APS neutrophils. Neutrophil extracellular trap (NET) release was assessed in static neutrophil-HUVEC cultures. RESULTS/ANTICIPATED RESULTS: Pre-treating control neutrophils with APS plasma resulted in increased adhesion as compared with control plasma (>2.5-fold for $n = 12$ plasma samples; $p < 0.05$). This was true under both venous conditions (low shear) and conditions representative of the microvasculature (pulsatile flow and higher shear). Control neutrophils treated with APS plasma demonstrated upregulation of CD64, CEACAM-1, beta-2 glycoprotein I, and activated Mac-1 on the neutrophil surface, as well as shedding of L-selectin. Upregulation of activated Mac-1 and shedding of L-selectin were also triggered by IgG purified from APS plasma. For these changes to be meaningful clinically, we reasoned that they should be present on neutrophils in the peripheral blood of APS patients. Indeed, perfusion of anticoagulated blood through the flow chamber resulted in increased adhesion of patient neutrophils as compared with controls (>5-fold for $n = 18$ patients; $p < 0.05$). Similarly, patient neutrophils demonstrated upregulation of CD64, CEACAM-1, beta-2 glycoprotein I, and activated Mac-1 on the neutrophil surface. A monoclonal antibody specific for activated Mac-1 reduced the adhesion of APS neutrophils to HUVECs in the flow-chamber assay (>2-fold reduction for $n = 5$ patients; $p < 0.05$). Importantly, the same monoclonal antibody reduced NET release in neutrophil-HUVEC co-cultures. DISCUSSION/SIGNIFICANCE OF IMPACT: APS neutrophils have an increased adhesive potential, which is dependent upon the activated form of Mac-1. This may lower the threshold for both neutrophil-endothelium engagement and NET release in patients, and thereby have implications for events such as venous thrombosis. Studies are underway to determine the extent to which Mac-1 is a viable therapeutic target in preclinical models of APS.

3114

Investigating the therapeutic potential of parthenolide in the treatment of hematopoietic neoplasms in dogs

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OBJECTIVES/SPECIFIC AIMS: Determine PTL's mechanism(s) of action in a panel of canine hematopoietic cell lines; this will enable us to 1) verify that PTL is working as expected and 2) rationally select combination therapeutics. Characterize the in vitro sensitivity of canine hematopoietic cell lines to PTL in combination with other

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chemotherapeutic agents. Determine immunohistochemical NFκB expression in tissue microarrays of spontaneous canine neoplasms and correlate with outcome-linked data. Characterize the in vivo sensitivity of canine hematopoietic cell lines to PTL using a murine xenograft model. METHODS/STUDY POPULATION: Growth inhibition assays were performed using a panel of canine mast cell, histiocytic sarcoma, lymphoma, and leukemia cell lines, with PTL alone or in combination with redox-perturbing standard-of-care therapeutics. Cell death was assessed using flow cytometry. Immunofluorescence and immunoblotting were used to assess NFκB localization and phosphorylation of NFκB p65 (transcriptional activation), respectively. Intracellular glutathione with and without PTL and combination chemotherapeutics will be assessed spectrophotometrically. Archived spontaneous canine tumors will be evaluated immunohistochemically (IHC) for increased NFκB pathway activation relative to normal control tissues. Nude mice will receive intravenous, intraperitoneal, or subcutaneous injections of canine HS cells and will be treated with PTL or with PTL in combination with standard-of-care chemotherapeutics. RESULTS/ANTICIPATED RESULTS: Results: All immortalized canine cell lines evaluated are sensitive to PTL therapy and undergo dose-dependent apoptosis following exposure to drug. PTL exposure leads to inhibition of NFκB, as evidenced by immunofluorescent nuclear exclusion and decreased p65 phosphorylation. Some chemotherapeutics appear to synergize with PTL in vitro. Anticipated results: We expect to find increased IHC NFκB pathway activation in malignantly transformed tissues relative to controls. We expect standard-of-care therapeutics to synergize with PTL in vivo based on preliminary in vitro data. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will determine whether PTL therapy may be beneficial in dogs with a variety of hematopoietic neoplasms, either alone or in combination with other therapeutics that are currently in clinical use. Dogs with mast cell or histiocytic neoplasia are an excellent model for rare and deadly human diseases, which may also benefit from PTL therapy.

3281

Management of Acute Rejection in Penile Allotransplantation

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OBJECTIVES/SPECIFIC AIMS: Objective: To summarize the diagnosis and management of two acute rejection (AR) episodes in the first penis transplant patient in the U.S. Background: Vascularized composite allotransplantation (VCA) has been utilized for state-of-the-art reconstruction of devastating craniofacial defects, limb loss, and recently, severe genitourinary defects. To date, more than 200 VCA's have been performed, of which four successful penis transplants have been achieved worldwide (Two in the U.S.). However, despite the technical success of VCAs in general, acute rejection episodes remain a significant postoperative management problem, with 80-85% experiencing at least one episode in the first-year post-transplantation. The incorporation of skin in VCAs, which is highly immunogenic, allows early visible recognition of rejection but requires prompt management to prevent allograft failure as well as the progression of chronic rejection, which has been associated with the frequency of acute rejection episodes in preclinical models. We present the first report of acute rejection in a penile allograft. AR episodes in VCA typically manifest with erythema of the allograft skin and/or maculopapular lesions that can be either patchy, focal or diffuse. Histopathologic assessment is essential for diagnosis and management. The Banff classification of histopathologic criteria for degree of AR is most commonly utilized to direct clinical management. METHODS/STUDY POPULATION: We reviewed the clinical course of the first American patient who underwent penis transplantation at Massachusetts General Hospital in 2016. Postoperatively, routine clinical and chemical assessment (immunosuppression levels, routine blood work) was performed, with increased frequency during AR episodes. Skin punch biopsies were obtained during (suspected) AR episodes, analyzed and graded according to the Banff 2007 classification of rejection of skin-containing composite allografts. Histopathologic tissue assessment included CD3, C4d, CD4/8, CD20 FOXP3 and cellular infiltration (hyper keratinization, lymphocytic infiltrate, dermal erosion, macrophage, eosinophilia, T-cell infiltration) and epidermal or perivascular fibrosis. RESULTS/ANTICIPATED RESULTS: The patient is a 65-year-old male with history of penile carcinoma requiring subtotal penectomy in 2012. He is currently 30-months post penile transplantation (as of 11/15/2018). First Rejection Episode: At 28 days post-transplantation, the patient noted induration, swelling and erythema of the allograft, which was diagnosed as AR clinically (Image 1A). Biopsy showed a Banff Grade III AR, with focal keratinocyte apoptosis with lymphocytic infiltration in epidermis and arteriolar endothelialitis with perivascular inflammation. Initially this episode was treated for 2 days with 2 pulse doses of methylprednisolone (500mg/d IV) with clinical improvement. However, recurrent allograft erythema was observed on postoperative day 32 and an acute rejection grade III according the Banff classification was confirmed by a second biopsy that demonstrated epidermal perivascular lymphocytic infiltrates, spongiosis and dyskeratosis, deep dermis focal lymphocytic infiltrates and focal infiltrates in arterioles as well as endothelialitis in venules. Donor specific antibodies and C4d were negative. CD3+ T cells were present in the epidermis and perivascular space. This was treated with anti-thymocyte globulin (thymoglobulin) course for 4 days (1.5mg/kg/day IV) and 3 more pulse doses of methylprednisolone (500mg/d IV.), followed by a prednisone (250mg/d) taper to baseline. This resulted in complete resolution of AR. Second Rejection Episode: At 10.8 months post VCA the patient presented with penile erythema and scrotal swelling suggestive of AR and received three doses of methylprednisolone (day 1: 500mg/d IV, day 2: 1000mg/d IV and day 3: 500mg/d IV respectively) followed by increased baseline prednisone (10mg PO daily; increased dose compared to previous AR episode). A skin biopsy confirmed Banff Grade III AR. Compared to the previous biopsy, this biopsy demonstrated an increased density of lymphocytic inflammation of the dermis with endarteritis. Prominent involvement of epidermis and adnexal structures corresponding to acute T-cell mediated rejection was also observed (Figure 1). Donor specific antibodies and C4d were again negative. Three doses of ATG (1.5mg/kg/day IV) were administered. In addition, tacrolimus was increased and local tacrolimus (1% ointment) treatment was begun. Clinical signs of rejection improved and repeat biopsy showed dramatic histopathological improvement. Current maintenance immunosuppressive regimen consists of tacrolimus, sirolimus, prednisone, mycophenolic mofetil acid (MMF), rapamycin, and tacrolimus ointment, with no new clinical or histopathological signs of rejection (Image 1B). DISCUSSION/SIGNIFICANCE OF IMPACT: We report the first described case of acute T-cell mediated rejection in penile transplantation. These rejection episodes demonstrated that, even on stringent immunosuppressive regimens, severe acute rejection episodes in VCA may still occur. Edema and acute induration

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