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

Gautam Sule[1], William J. Kelley[3], Srilakshmi Yalavarthi[2], Omolola Eniola-Adefeso[3] and Jason S. Knight[1]
[1]University of Michigan School of Medicine; [2]Department of Internal Medicine, University of Michigan and [3]Department of Chemical Engineering, University of Michigan

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

Lisa J Schlein, ACVP[1], Aubree Peterson[2], Barbara Rose[2] and Douglas Thamm[2]
[1]University of Colorado at Denver and [2]Colorado State University

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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