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DISSERTATION

Regulation and Dysregulation of the Anti-Viral Response by Human
Plasmacytoid Dendritic Cells

Part 1: Investigating the Role of Epigenetic Modifiers in pDC Function

Part 2: Investigating How SARS-CoV-2 Infection Modifies pDC Number,
Phenotype and Function

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Medical Science Building C600

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PART 1: ABSTRACT

In response to viruses, plasmacytoid dendritic cells (pDC) are potent producers of IFN- α and can mature into antigen-presenting cells. In vitro, virus-stimulated pDC are defined as IFN- α producing cells after 6 hours and antigen-presenting cells after 24 hours. Our lab previously identified that pDC become dysfunctional and develop an altered phenotype in the chronic virus infection, HIV-1. In this study, we investigated the role of DNA methyltransferase1 (DNMT1) and Ten-eleven translocation methyl-cytosine dioxygenase 1, 2, and 3 (TET1, TET2 and TET3) in pDC activation. DNMT1 gains a methyl group from S-adenosyl methionine and transports the methyl group to the genome, while TETs utilize α -ketoglutarate from the TCA cycle, oxidize and remove methyl groups. Adding a methyl group silences gene expression while removing a methyl group is known to activate gene expression. In murine pDC, a zinc finger protein, CXXC5, recruits TET2 near the IRF-7 promotor region to maintain genome hypomethylation and promote constitutive high protein levels of IRF-7 in pDC. The ablation of CXXC5 and TET2 disrupts IRF-7 expression and IFN- α production and promotes hypermethylation. To determine the role of the TET family (TET1-3) and DNMT1 in human pDC function, we freshly isolated PBMC from healthy donors and stimulated with Herpes Simplex Virus (HSV) or Influenza-A virus (IAV) for 6- and 24 hours. After 6 hours, TET3 was distinctively induced in IFN- α producing compared to IFN- α non-producing pDC. The TET3 inhibitor, DMOG, dampened virus-stimulated pDC IFN- α production more efficiently than the TET1/2 inhibitor, Bobcatt339. We used an IRAK inhibitor to determine that TET3 is acting downstream of IRAKs while TET2 is acting upstream. To confirm the essential role of TET3 in virus-stimulated pDC IFN- α production, we knocked down TET3 in HSV-stimulated pDC and observed pDC dysfunction. TET3-negative but not TET3-positive pDC were unable to produce IFN- α . We inhibited the function of DNMT1 by reducing methionine levels in media and observed induction of IFN- α production. The overexpression of DNMT1 silenced pDC function. In conclusion, TET3 and DNMT1 are essential in regulating human peripheral virus-stimulated pDC IFN- α production.

PART 2: ABSTRACT

Plasmacytoid dendritic cells (pDC) are innate immune cells and potent interferon-alpha (IFN- α) producers. In chronic viral infections, as in HIV infection, pDC are low in number and function, contributing to disease progression. Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2), an RNA virus that causes Coronavirus Disease 2019 (COVID-19), has taken over one million lives in the United States. This study evaluated pDC function and phenotype from hospitalized patients with COVID-19 treated with or without dexamethasone (DEX). DEX relieves inflammation and has been shown to suppress pDC function and differentiation and enhance apoptotic death. We isolated peripheral blood mononuclear cells (PBMCs) from hospitalized COVID-19 patients and healthy and convalescent controls. Flow cytometric analysis of steady-state COVID-19 patients' pDC compared to healthy controls' pDC disclosed that the co-stimulatory molecule, CD86, migratory molecule, CCR7, and cellular proliferation marker, Ki-67, are more highly expressed while the transcription factor, IRF-7, was reduced in the patient vs. healthy controls. This phenotype describes newly released pDC circulating in COVID patients and that some pDC are partially matured and preparing to migrate to the lymph nodes. In the same donors, PBMCs were stimulated with either Influenza-A (IAV) or Herpes Simplex Virus -1 (HSV), and pDC IFN- α and TNF- α production were assessed. pDC from the patients were deficient in IFN- α and TNF- α

production, but pDC from DEX-treated patients were even more compromised, thus demonstrating that pDC dysregulation in COVID-19 is independent of DEX treatment. Furthermore, the percentage of IFN- α + pDC was inversely correlated with disease severity, and pDC numbers and function recovered in convalescent patients. Circulating IFN- α was elevated in plasma from patients with mild/moderate COVID-19 but reduced in patients with severe COVID-19. Low IFN- α levels correlated with disease progression. Using the senescence marker, senescence-associated β -galactosidase (SA- β Gal), we determined that pDC from COVID-19 patients were more senescent in males than in female patients, which aligns with higher mortality in male than female patients. We conclude that COVID-19 patients' pDC are more senescent in males, presumably newly formed, partially matured, dysfunctional, and likely contribute to SARS-CoV-2 pathogenesis.