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Characterizing the PolyIC-induced activation and apoptosis of MuTuDCs by determining the influence of Bim isoforms and Corticosterone

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By

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Abstract

Dendritic cells (DCs) are a critical subset of immune cells responsible for connecting the innate and adaptive immune response. Dendritic cells are activated by exposure to Pathogen Associated Molecular Patterns (PAMPs). Polyinosinic:polycytidylic acid (PolyIC) is synthetic double-stranded RNA that functions as a PAMP. Corticosterone (CORT), a stress hormone, is generally immunosuppressive. The response of murine DCs (MuTuDC) to PolyIC and CORT exposure is being investigated. ELISA demonstrated that PolyIC exposure increased IL-12 production, an indicator of DC activation. Increased cell death was also observed following PolyIC exposure, but pre-treatment with CORT decreased cell death. The literature suggests that DC death post activation is important for regulating the immune response. However, the pathway mediating PolyIC-induced DC death and the influence of CORT on the process is loosely characterized. Literature suggests that apoptosis occurs through the pro-apoptotic protein Bim. It is hypothesized that CORT is altering Bim isoform expression to reduce cell death. Bim has three predominant isoforms, varying in function, that regulate apoptosis. Primers derived from the literature for both murine and human Bim mRNA isoforms were analyzed using the NCBI BLAST program to ensure detection of the three isoforms. However, detection of Bim protein and mRNA isoforms in MuTuDCs was difficult. Therefore, Human-Diploid Fibroblast cells immortalized with T-antigen (HDF+T) were used to establish necessary RT-PCR and Western protocols. After demonstrating Bim isoform(s) presence in HDF+Ts, future studies will involve MuTuDCs pretreatment with CORT and then treatment with PolyIC, or another apoptosis inducer such as 5-fluorouricil (5-FU). Elucidating the PolyIC-mediated cell death pathway and the impact of CORT will provide novel information about factors influencing DC abundance and lifespan, which ultimately influence the immune response.

Introduction

The immune system is complex, involving the coordination of various cell types to ultimately prevent infection and tumor formation. The immune system is comprised of two main branches: the innate and adaptive system. The innate system is the first line of defense against foreign particles, rapidly eliciting the same initial response against the identified foreign ligand each time. The adaptive system takes longer to develop upon initial exposure, but affords incredible specificity and memory against the foreign ligand. To elicit a successful immune response, communication and coordination between the innate and adaptive system is critical (Murphy & Weaver, 2017).

Dendritic cells (DCs) are potent antigen presenting cells responsible for connecting the innate and adaptive immune system. While there are two types of DCs, it is the conventional DCs that are essential to bridge the two branches of the immune system via their ability to communicate with T-cells, cells of the adaptive immune system, following their activation and maturation. Immature DCs are located in peripheral tissues and are highly phagocytic, continuously sampling their surrounding environment (Banchereau et al., 2000). DCs become activated by certain pathogenic ligands, called Pathogen Associated Molecular Patterns (PAMPS). PAMPs are specific, highly conserved molecules of a pathogen. Several synthetic PAMPs, such as the viral double-stranded RNA mimic: PolyIC, have been created. PAMPs are recognized by DCs receptors: Pattern-Recognition Receptors (PRR). Toll-like receptors (TLR) are one predominant class of PRR that are responsible for recognizing and binding different PAMPs. For example, TLR-3 is responsible for recognizing viral double-stranded RNA. Following TLR-3 binding to PolyIC, intracellular signaling occurs that functions to activate the DC via changes in protein expression and protein secretion. Specifically, activation is marked by

upregulation of MHC I, MHC II, CD80, and CD86 proteins as well as increased secretion of IL-12 (Murphy & Weaver, 2017). Activation causes immature DCs to mature. Mature DCs then migrate to secondary lymphoid organs, where they can activate T-cells by presenting a portion of the pathogen on MHC I or MHC II along with co-stimulatory signals (Banchereau et al., 2000).

Mature DC, resulting from activation, are critical for eliciting an adaptive immune response. However, it is known and has been observed that DC activation is often followed by cell death, serving to regulate the immune response (Kushwah & Hu, 2010). There are various forms of cell death. Literature suggest that apoptosis, programmed cell death, occurs post activation (Kushwah & Hu, 2010). Apoptosis can be elicited via both intrinsic and extrinsic pathways. Extrinsic apoptosis pathways can be initiated by Tumor Necrosis Factor Receptor family proteins, like Fas, which activate caspase 8 to trigger a series of intracellular caspase protein signaling, causing apoptosis (Maracco et al., 2011). Intrinsic apoptosis involves numerous outer surface mitochondrial proteins, the Bcl-2 family proteins, that initiate a caspase cascade to cause apoptosis (Maracco et al., 2011). The Bcl-2 family protein are largely responsible for regulating intrinsic apoptosis. The BH3-only proteins, a subgroup of the Bcl-2 family, are responsible for promoting apoptosis (Chen, et al., 2007). It has been demonstrated by Maracco et al. that MuTuDC undergo intrinsic apoptosis following PolyIC-mediated activation (2011). The observed intrinsic apoptosis was mediated by BH3-only proteins, specifically Bim.

Bim, a pro-apoptotic BH3-only protein, has been implicated in DC death. In healthy cells, Bim remains in an inactive conformation until it receives signals that promote structural changes resulting in its active conformation (Marani et al., 2002). In DCs, signaling from TLRs causes an upregulation of Bim, which likely initiates apoptosis promotion (Sionov et al., 2015). Unsurprisingly, Bim-deficient DCs in mice display decreased cell death following activation

(Chen et al., 2007). Stress hormones called glucocorticoids, such as corticosterone (CORT), are generally immunosuppressive and have also been found to induce production of Bim in other immune cells (Sionov et al., 2015). Specifically, glucocorticoids can upregulate Bim expression and influence the ratio of pro- and anti-apoptotic proteins (Gruver-Yates & Cidlowski, 2013). There are numerous Bim protein isoforms that result from differential splicing of Bim mRNA, creating mRNA isoforms varying in exon composition and consequently function (Marani et al., 2002). Three predominant Bim isoforms have been identified and completely sequenced in both humans and mice: Bim Extra Long (BimEL), Bim Long (BimL), and Bim Short (BimS). While the three main Bim isoforms vary in size and function, they are all associated with the mitochondria and form heterodimers with other anti-apoptotic and pro-apoptotic Bcl-2 proteins to ultimately promote apoptosis (UniProt, 2020). In humans and mice, BimS has been identified as the most potent inducer of apoptosis while BimEL is the least potent (UniProt, 2020). Given their varying potency, Bim isoform presence and the proportions of the three isoforms likely contribute to cell death determination.

Studying DCs *in vivo* has been challenging due to their low relative abundance (Maracco et al., 2012). Maracco et al. modified murine splenic DC to develop a novel immortalized DC cell line that mimics naturally occurring CD8+ DCs (MuTuDC), enabling DC study *in vitro* (2012). CD8+ DC are a subset of DCs, characterized by their surface protein expression, that have been identified as the best for communicating with T-cells. MuTuDCs were obtained by Dr. Lancaster from Dr. Cresswell's lab at Yale University. Using MuTuDCs, our lab has previously demonstrated PolyIC-induced activation followed by PolyIC-mediated cell death. Interestingly, our lab found that CORT pretreatment reduced PolyIC-mediated MuTuDC cell death. Literature implicates Bim in DC apoptosis, but detection of Bim in MuTuDCs in our lab has been difficult.

Therefore, current studies focused on using Human-Diploid Fibroblast cells immortalized with T-antigen (HDF+T) to establish necessary protocols. MuTuDCs will then be used to investigate the role of Bim isoforms and CORT on PolyIC-mediated cell death. It is hypothesized that CORT is altering Bim isoform expression to reduce PolyIC-mediated MuTuDC cell death. Determining the impact of Bim isoforms and CORT on the PolyIC-mediated cell death pathway will provide new information about factors influencing DC abundance, lifespan, and their ultimate role in the immune response.

Methods and Material

Culture conditions and cell maintenance. MuTuDCs were previously obtained by Dr. Lancaster from Dr. Cresswell's lab at Yale University. The MuTuDCs were cultured in Gibco Iscove's Modified Dulbecco's Medium-glutamax base media supplemented with 10% Fetal Bovine Serum, 2%* sodium bicarbonate, 50ug/mL penicillin-streptomycin, 10mM Hepes, and 50uM beta-mercaptoethanol. MuTuDCs were incubated loose lid at 37℃ and 5% CO_2. MuTuDCs were routinely split using a versene solution (200mg/L EDTA in PBS) every 2-3 days, depending on confluency, at a 1:3 split ratio. The HDF+T clone 1 cells were obtained from Dr. Cavender's lab at Elizabethtown College. The HDF+Ts were grown in Dulbecco's Modified Eagle Medium supplemented with 10% Fetal Bovine Serum, 3%* sodium bicarbonate, and 1% penicillin-streptomycin. HDT+Ts were incubated loose lid at 37℃ and 5% CO_2. HDF+Ts were typically split every 3-4 days using a 1:10 trypsin-versene solution following two rinses with PBS at a 1:5 ratio.

Primer synthesis. Primers for human and murine Bim mRNA were identified in the literature: human forward (5'-GGCAAAGCAACCTTCTGATG-3') and reverse (5'-AGACCACCCACGAATGGTTA-3') (Anczuków et al, 2012); murine forward (5'-CTGAGTGTGACAGAGAAGGTGG- 3') and reverse (5'-ACCGCGAGGCTGAAGACCAC-3') (Chen et al., 2007). The primers were extensively analyzed using the NCBI BLAST program to ensure differential detection of each of the three main isoforms of Bim. Corresponding expected band sizes for each isoform were determined. The primer sequences were synthesized by Eurofins Lancaster Laboratories, PA. Appropriate Millipore water volumes needed to rehydrate the primers to 100uM were added. Primer 100uM stocks were diluted 1:10 with

Millipore water to make 10uM working stocks. Primers for human and murine actin mRNA were also obtained to serve as a positive control, in addition to T-antigen primers.

Cell treatments. Prior to RNA isolation, HDF+Ts and MuTuDCs were untreated or treated with PolyIC or 5-flurouricil (5-FU). Cells were treated with 5ug/mL PolyIC for 12 hours and treated with 5ug/mL* 5-FU for 2 hours prior to RNA isolation. Cells in each treatment group were seeded at the same time with the same number of cells.

RNA isolation. RNA was isolated using QIAGEN RNeasy Mini Kit (QIAGEN Cat No. 74104). Cells were treated, grown to approximate confluency in a T25, then harvested following their typical procedure. A working solution of 10% harvested cell suspension in 90% trypan exclusion dye totaling 100uL was made and 12uL were loaded into a hemocytometer to determine the cell count. The remaining cells were centrifuged at 1200rpm for 2 minutes to obtain a cell pellet. The cell pellet count dictated the appropriate volume of Buffer RTL subsequently added directly to the pellet. One volume of 70% ethanol was then added and mixed well. 700uL of this mixture was transferred to an RNeasy Mini spin column inside a 2mL collection tube. The column was centrifuged for 15 seconds (s) at 8000x *g*; the flow-through was discarded. Next, 700uL of Buffer RW1 was added to the column, which was then centrifuged for 15s at 8000x *g*; the flow-through was discarded. 500uL of Buffer RPE was added to the column, which was then centrifuged for 15s at 8000x *g*; the flow-through was discarded. Another 500uL of Buffer RPE was added to the column, which as then centrifuged for 2 minutes at 8000x *g*; the flow-through was discarded. The RNeasy spin column was placed in a 1.5mL collection tube and 40uL of RNase-free water was added directly to the member. The column was centrifuged for 1 minute at 8000x *g*. The eluted RNA was located in the collection tube. The RNA isolates were analyzed with a nanodrop instrument to determine the RNA concentration and stored at 4℃.

cDNA synthesis. Isolated RNA was used to create cDNA using Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR. Reagents and RNA were thawed on ice and gently mixed prior to use. A mix totaling 20uL was created using 4uL 5x reaction mix, 2uL maxima enzyme mix, 4uL* Template RNA, and 10uL of nuclease-free water. The mix was gently centrifuged then placed in a thermocycler programed for 10 minutes at 25℃, then 15 minutes at 50℃, and ending with 5 minutes at 85℃. The cDNA products were analyzed with a nanodrop instrument to determine the DNA concentration and stored in at 4℃.

Polymerase Chain Reaction. cDNA from different cell treatment groups in conjunction with Bim, actin, and T-antigen primer pairs were used with the Invitrogen Platinum II Hot Start PCR 2x Master Mix kit (Cat No. 14000-013). Master mixes for n+1 50uL reactions were created for each primer-cDNA pair. The master mixes contained 25uL Platinum Hot Start PCR 2x Master Mix, 1uL forward primer (10uM), 1uL revere primer (10uM), and 22uL nuclease-free water per reaction. Aliquots of 49uL of master mix were distributed into PCR tubes, then 1uL of cDNA from the three treatments groups (untreated, PolyIC treated, 5-FU treated) was added accordingly. One reaction per experiment used water instead of cDNA to serve as a negative control. The actin-cDNA and T-antigen-cDNA pairings served as positive controls for all treatment groups. Once each reaction component was added, PCR tubes were briefly centrifuged and placed into the thermocycler when it reached 90℃. The thermocycler was programmed as follows: 94℃ for 2 minutes; 94℃ for 14s, 60℃ for 15s, 68℃ 15s, repeated for 31 total cycles; 4℃ hold. Upon program conclusion, each PCR tube was centrifuged briefly. From each PCR tube, 2uL of product was taken and added to 10uL of 6x loading dye. A 1.5% agarose in 1x TBE gel was prepared with ethidium bromide. Once the gel was solidified, it was placed in the electrophoresis apparatus and completely overlaid with 1x TBE. A TrackIt Invitrogen 100bp

DNA ladder or pGEM ladder and all 12uL of each prepared product sample were loaded into the gel. The corresponding loading order was recorded. Gels were run at approximately 130 volts for 30 minutes. Gels were then imaged using the camera available in the Biology Department, saved to the Biology Department computer, and labelled appropriately.

Statistical Analysis. Microsoft Excel was utilized to analyze all cell activation and cell death data. For activation and cell death, averages with corresponding standard deviations were calculated. Data should be analyzed using ANOVA.

*estimated.

Results

PolyIC exposure induced MuTuDC activation. Flow cytometry and ELISA previously performed as part of SCARP 2018 demonstrated MuTuDC activation following PolyIC treatment (data not shown). Flow cytometry demonstrated a marked upregulation of MHC I and CD80 proteins following PolyIC exposure, and ELISA demonstrated increased secretion of IL-12 following PolyIC exposure. Both are indicative of DC activation. It was observed that IL-12 secretion was not dependent on PolyIC dose; MuTuDCs treated with anywhere from 0.5ug/mL to 10ug/mL PolyIC exhibited approximately the same average amount of IL-12 secretion (data not shown). IL-12 secretion was dependent on PolyIC exposure time, as seen in **Figure 1**.

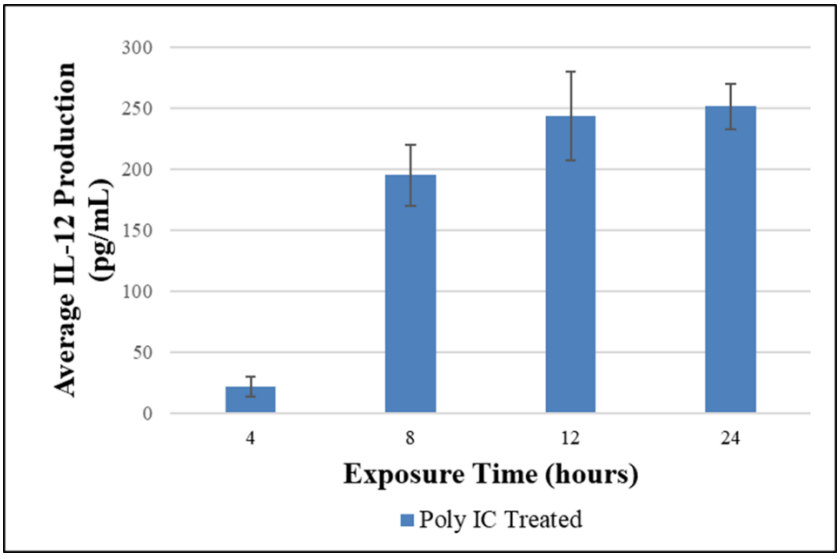

Figure 1. Average MuTuDC IL-12 production over time. MuTuDCs (n=3) were treated with 5ug/mL PolyIC for varying times. IL-12 production was quantified via ELISA and averages were graphed with respective standard deviations. Average IL-12 production markedly increased between 4 hours and 8 hours of PolyIC exposure, then stabilized around 12 hours of exposure. Control, untreated MuTuDCs did not produce any IL-12. Data are representative of multiple experiments.

PolyIC exposure resulted in MuTuDC death. While MuTuDC activation following PolyIC exposure was demonstrated, MuTuDC cell death was observed following PolyIC treatment via trypan exclusion dye. Cell death was not dose dependent but was dependent on exposure time. MuTuDCs treated with anywhere from 1ug/mL to 10ug/mL PolyIC exhibited approximately 50% cell death. However, longer PolyIC exposure time resulted in increased MuTuDC cell death, as shown in **Figure 2**. Interestingly, it was observed that pre-treatment of MuTuDC with CORT before PolyIC treatment markedly reduced cell death (**Figure 3).**

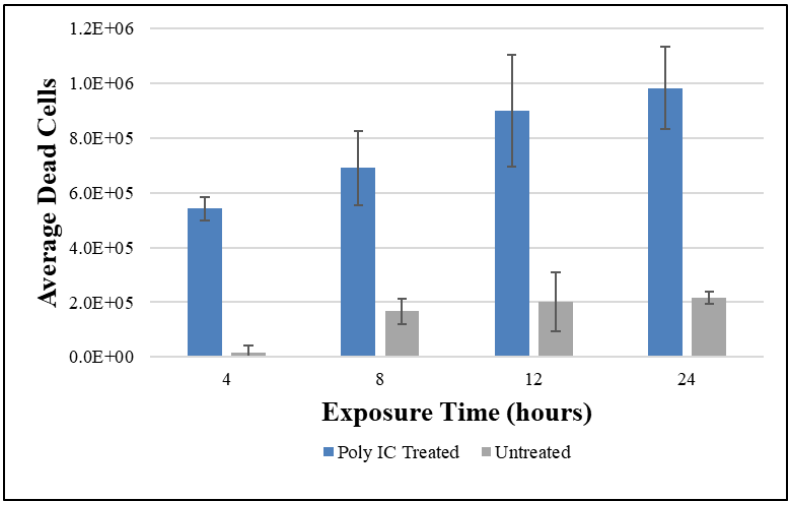

Figure 2. Average MuTuDC cell death over time. MuTuDCs (n=3) were treated with 5ug/mL PolyIC or nothing for varying times. Cell death was quantified using trypan exclusion dye and averages were graphed with respective standard deviation. Average MuTuDC PolyIC-mediated cell death gradually increased over time. Control, untreated MuTuDCs exhibited little cell death over time. Data are representative of multiple experiments.

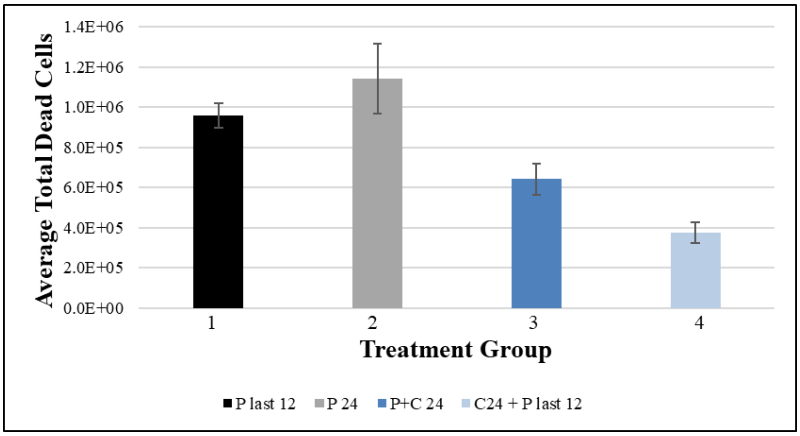

Figure 3. Average total dead MuTuDCs after four different treatments. MuTuDC (n=3) were treated with 5ug/mL PolyIC and/or $1x10^{-6}$M CORT for varying times. Total dead cells were counted and graphed with respective standard deviation. Treatment 1 represents 12 hrs of PolyIC treatment. Treatment 2 represents 24 hrs of PolyIC treatment. Treatment 3 represents 24 hrs of PolyIC and CORT treatment simultaneously. Treatment 4 represents a 12 hr pretreatment with CORT followed by 12 hrs of PolyIC treatment. Data are representative of multiple experiments.

Bim protein detection in MuTuDCs is difficult. Literature suggests that the protein Bim facilitates DC cell death (Marraco et al., 2011). Preliminary studies conducted during the Fall 2018 semester to establish Bim presence in MuTuDCs demonstrated that Bim protein

detection is difficult. A western blot probing for Bim was conducted to investigate if intrinsic apoptosis was occurring in the MuTuDCs following PolyIC treatment. Bim protein was not detected via western blot in MuTuDC treated with PolyIC or in untreated, control cells (data not shown). Another method for detecting proteins is immunofluorescence (IF). IF was performed for Bim, but Bim was again not detected (data not shown).

Primer analysis indicates effective detection. Due to Bim protein detection difficulties, Bim mRNA detection was pursued. Primers for mouse and human Bim mRNA were identified in the literature (Anczuków et al, 2012; Chen et al., 2007). The primer sequences were analyzed using NCBI BLAT Global Align program to verify differential detection of the three main Bim isoforms, the target transcripts. First, preliminary analysis of variations in Bim isoforms sequences, due to variable exon inclusion, was performed. Complete sequences for each main human and murine Bim isoform were obtained using NCBI BLAST and compared using NCBI Global Align program to determine their relative size as well as shared exon sequences (**Figure 4 & 5**). The main three human and murine Bim isoforms vary slightly in nucleotide sequences, making it essential for the primers to be exclusively used for their corresponding species. It was determined that the three main Bim isoforms vary in size as follows: BimEL > BimL > BimS. Each of the main human and murine Bim isoforms contain the same beginning and ending sequences. To confirm that the primers were designed for shared Bim isoform sequences, NCBI Global Align was used to determine the primer to isoform alignment (**Figure 4 & 5**). Once the primer alignments were established, expected band sizes for each Bim isoforms corresponding PCR product were calculated. Expected human Bim isoform band sizes are as follows: BimEL= 545 base pairs (bp), BimL=365bp, BimS= 275bp. Expected murine Bim isoform band sizes are as follows: BimEL= 500bp, BimL= 332bp, BimS= 242bp.

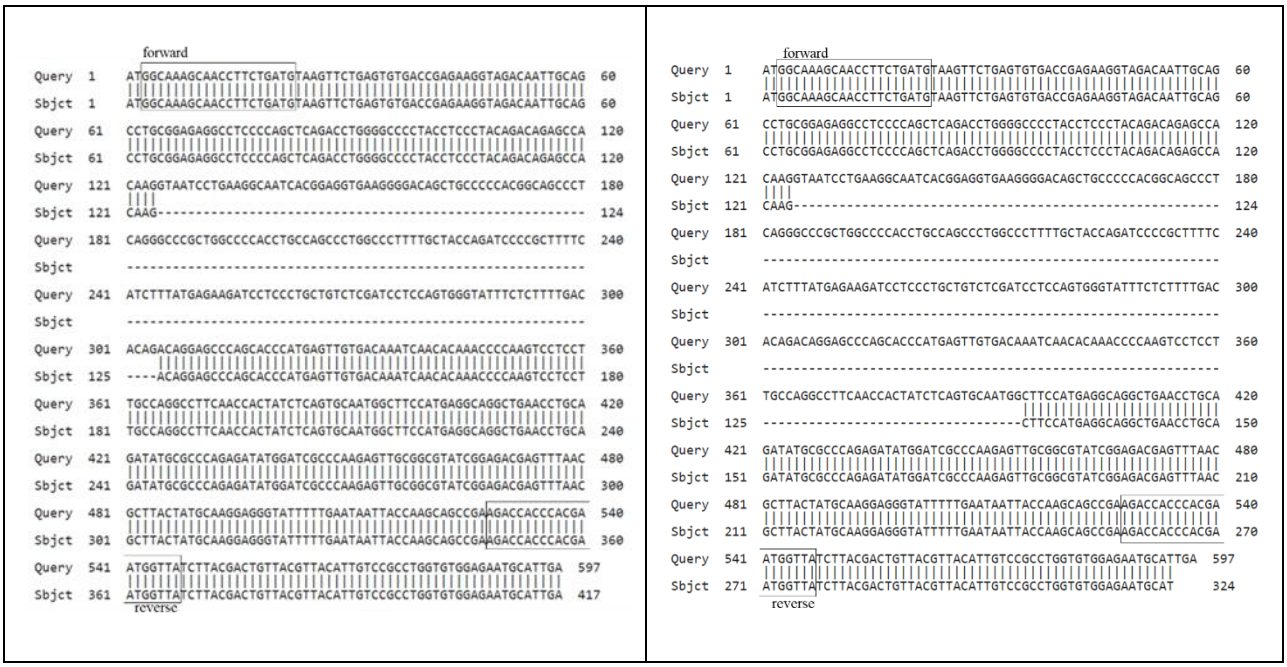

Figure 4. NCBI Global Align results for main human Bim isoforms and primers. Complete human cDNA sequences for the main three Bim isoforms were obtained and aligned with the human Bim primers. The left image is the alignment of the BimEL and BimL isoforms; the right image is the alignment of the BimEL and BimS isoforms. BimEL is the top sequences in both images. The boxed regions indicate forward and reverse primer alignments.

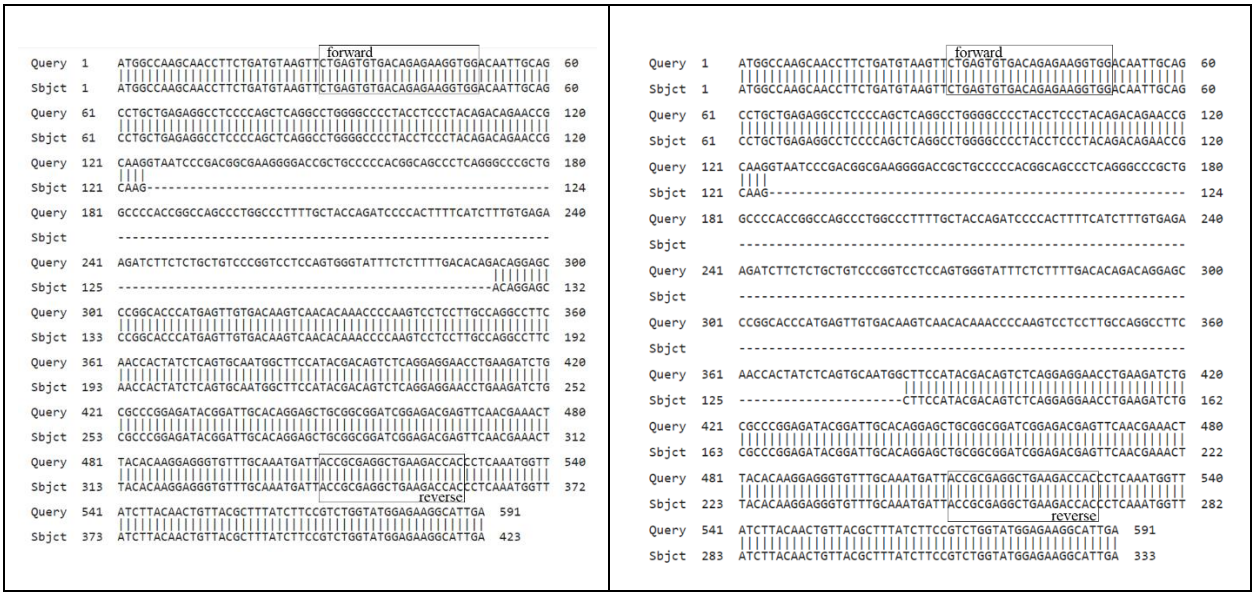

Figure 5. NCBI Global Align results for main murine Bim isoforms and primers. Complete murine cDNA sequences for the main three Bim isoforms were obtained and aligned with the murine Bim primers. The left image is the alignment of the BimEL and BimL isoforms; the right image is the alignment of the BimEL and BimS isoforms. BimEL is the top sequences in both images. The boxed regions indicate forward and reverse primer alignments.

Bim isoform presence was observed across all treatments. Using the verified primers, PCR following cDNA synthesis was conducted. However, initial experiments did not detect Bim mRNA isoforms in MuTuDCs (**Figure 6**). Due to detection difficulties, HDF+T cells were used to optimize the experimental parameters. RNA harvested from untreated, PolyIC treated, and 5-FU treated HDF+Ts was made into cDNA and used to perform PCR for Bim. Further trouble shooting entailed obtaining a new PCR kit to avoid any prior contamination that could have occurred with the originally shared PCR kit, adjusting annealing temperatures, placing the PCR tubes into the thermocycler at higher temperatures, using T-antigen as an additional positive control, and verifying the integrity of the RNA, cDNA, and primers. After extensive trouble shooting to optimize the experiment, a successful PCR gel for Bim in HDF+Ts was produced (**Figure 7**). All T-antigen and actin positive controls produced bands of the correct expected size. Water in the T-antigen group served as a negative control. Bands corresponding to BimEL (545bp) and BimL (365bp) were detected in all treatments, but BimS (265bp) was not detected. Bim band intensity varied slightly across the various treatments. The untreated group produced the most intense bands.

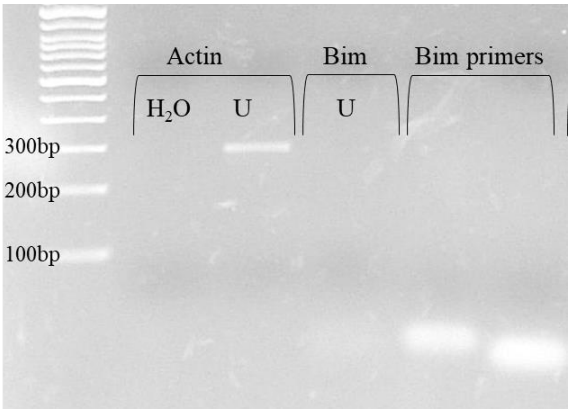

Figure 6. PCR for Bim in MuTuDCs. PCR was performed using untreated (U) MuTuDC cDNA harvested after various treatments. Actin served as positive controls. The water in the actin group served as a negative control. No bands were observed for Bim although Bim primers are present.

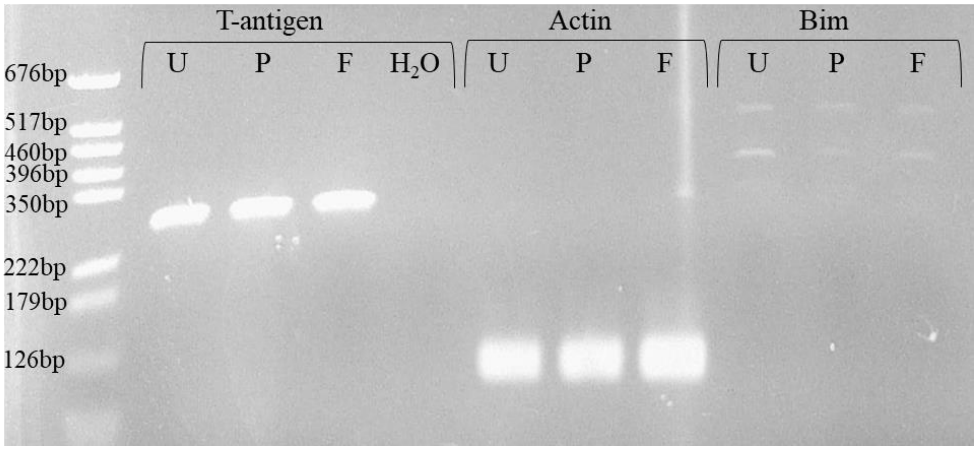

Figure 7. PCR for Bim in HDF+Ts after different treatments. PCR was performed using HDF+T cDNA harvested after various treatments. U represents untreated HDF+Ts. P represents HDF+Ts treated with 5ug/mL PolyIC for 12 hours. F represents HDF+Ts treated with 5ug/mL* 5-FU for 2 hours. T-antigen and actin served as positive controls. The water in the T-antigen group served as a negative control. Two bands were observed for Bim across all treatments.

After optimizing and successfully detecting Bim in HDF+Ts, PCR for Bim in MuTuDCs was performed successfully (**Figure 8**). All T-antigen and actin positive controls produced bands of the correct, expected size. Water in the T-antigen group served as a negative control. It is difficult to determine if the top Bim band in all the treatment groups corresponds to BimEL (500bp) or BimL (332bp), but it is likely that is corresponds to BimL. Bim band intensity varied slightly across the various treatments, with the PolyIC treatment producing the most intense bands. A faint, lower band in the PolyIC treatment group was also observed; it is again difficult to differentiate which Bim isoform it represents.

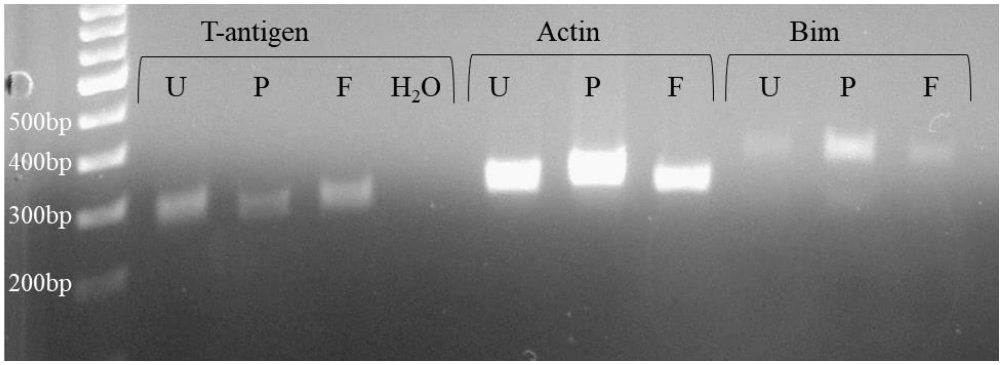

Figure 8. PCR for Bim in MuTuDCs after different treatments. PCR was performed using MuTuDC cDNA harvested after various treatments. U represents untreated MuTuDCs. P represents MuTuDCs treated with 5ug/mL PolyIC for 12 hours. F represents MuTuDCs treated with 5ug/mL* 5-FU for 2 hours. T-antigen and actin served as positive controls. The water in the T-antigen group served as a negative control. One to two bands were observed for Bim across all treatments.

Discussion

Proper regulation of immune responses is essential to avoid immunosuppression as well as autoimmunity. Dendritic cells play a crucial role in the development of immune responses because they connect the innate and adaptive immune responses. The intricate balance between DC activation and apoptosis serves to modulate immune responses. DC activation is necessary to elicit a strong enough adaptive immune response to facilitate the effective clearance of foreign particles, such as pathogens, to avoid immunosuppression (Murphy & Weaver, 2017). However, prolonged DC activation can result in autoimmunity and immune system induced pathology, demonstrating the necessity of DC clearance via apoptosis following activation (Chen et al., 2007). The balance between DC activation and apoptosis thus plays a critical role in immune response regulation.

Regulation of DC homeostasis via apoptosis is imperative, as DC lifespan influences the duration of lymphocyte stimulation, activation, and the subsequent immune response (Chen et al., 2007). Regulation of DC apoptosis occurs through multiple pathways. While all the specific genes involved in DC lifespan regulation have yet to be completely identified, Bim has been implicated as a critical protein for eliciting intrinsic DC apoptosis following activation (Marraco et al., 2011). Normally, Bim is expressed at low levels in DCs and is transcriptionally upregulated following TLR signaling. However, both Bim under- and over-expression adversely impact the immune response (Chen et al., 2007). Mice with Bim deficiencies in DCs demonstrate decreased DC cell death while simultaneously inducing hyperactivation of T-cells and increased autoantibody production, resulting in autoimmune diseases (Chen et al., 2007). When exposed to PolyIC specifically, Bim knockout mice exhibited significantly less DC cell death than their wild type counterparts (Chen et al., 2007). These results are corroborated by findings from Maracco et

al., which implicated Bim in conjunction with other BH3-only proteins in DC apoptosis following PolyIC induced activation (2011). Evidently, Bim is an important mediator of DC apoptosis and, consequently, lifespan.

Consistent with Maracco et al.'s findings, our lab demonstrated that PolyIC was a strong inducer of MuTuDC activation and subsequent MuTuDC cell death (2011). While our lab was able to detect Bim isoforms, Bim isoform quantification was not performed. Thus, it is currently indeterminant, based on our results, what affect PolyIC treatment has on Bim expression in MuTuDCs. Our results generally suggest that PolyIC may increase BimL isoform expression. Maracco et al. has previously demonstrated Bim upregulation in MuTuDCs following PolyIC treatment (2011). The impact of variable Bim isoform presence is currently unknown. Bim isoforms vary in potency of apoptosis induction (Marani et al., 2002). BimS is the most potent inducer of apoptosis, but was not definitively detected in either HDF+Ts or MuTuDCs across all treatment types; although, the utilized primers were demonstrated to be effective at detecting BimS based on NCBI sequence analysis. While it was evident that BimEL and BimL isoforms were present in HDF+Ts, it was harder to determine which Bim isoform the observed Bim band corresponded to, although it is likely BimL. Since Bim isoforms vary in apoptotic induction potency, further investigating the variations in Bim isoform presence and amounts in MuTuDCs could explain the observed differences in cell death following PolyIC treatment.

Given the importance of Bim in DC activation and apoptosis, glucocorticoids' ability to alter Bim expression is noteworthy (Gruver-Yates & Cidlowski, 2013). Generally, glucocorticoids are used for their anti-inflammatory and immune-modulatory properties, conferred by their ability to cause DC apoptosis via intrinsic apoptosis (Heidari et al., 2012). Thus, glucocorticoids' ability to upregulate Bim expression as well as influence the pro- to anti-

apoptotic protein ratios is logical and significantly influences the immune responses (Gruver-Yates & Cidlowski, 2013). However, our lab observed that pretreatment with CORT, a glucocorticoid, reduced MuTuDC cell death following PolyIC activation. Since Bim is largely regulated through transcription, it is possible that CORT is altering Bim isoform production and ratios to influence DC cell death (Heidari et al., 2012). DC maturation state may likely influence the potential CORT and Bim isoform interaction (Cao et al., 2013). Cao et al. found that glucocorticoid-mediated cell death only occurs in mature DCs, not immature DCs (2013). Our lab's observed enhanced MuTuDC survival with CORT pretreatment could be occurring as a result of glucocorticoids suppression of DC maturation, marked by glucocorticoid inhibition of antigen processing and presentation in DCs (Cao et al., 2013). Thus, our findings that pretreatment of CORT reduced cell death is consistent with the literature.

Due to DCs pivotal role in regulating immune responses, DCs are key targets for vaccine development (Trumpfheller et al., 2008). Many vaccines utilize PAMPs as adjuvants to enhance vaccine efficacy (Maracco et al., 2011). PolyIC has been identified as a promising adjuvant target for vaccines against intracellular pathogens (Hafner et al., 2013). While preliminary studies have found that PolyIC can strongly drive cell-mediated immunity, further safety and efficacy studies need to be conducted before it can be more widely utilized in a clinical setting (Hafner et al., 2013). Some preliminary studies using murine models have demonstrated that PolyIC is an effective adjuvant for vaccines targeting DCs (Trumpfheller et al., 2008). Conferring a better understanding of the impact of PolyIC, as well as CORT, on Bim isoforms in DCs could therefore serve to enhance vaccine development and efficacy.

Conclusions and Future Directions

A balance between DC activation and apoptosis is critical to develop an effective immune response while avoiding autoimmunity. PolyIC induces MuTuDC activation followed by MuTuDC cell death. Literature indicates that apoptosis following PolyIC activation involves the protein Bim. Bim detection has proven difficult, but after experimental optimization with HDF+Ts Bim isoforms were observed in MuTuDCs across all treatment groups via PCR. However, PCR is only qualitative, not quantitative. In the future, qPCR will be performed to quantitatively demonstrate variations in Bim isoform transcription resulting from PolyIC as well as CORT treatments. Due to unforeseen scheduling changes, investigation of the impact of CORT on Bim isoforms was not conducted. Given glucocorticoids' ability to alter apoptotic protein transcription, it is imperative to explicate the role of CORT in the PolyIC-mediated MuTuDC cell death pathway. This will provide novel information about factors influencing DC abundance and lifespan, which will improve the current understanding of the intricate balance required to elicit an effective immune response as well as potentially improve vaccine efficacy for vaccines involving PolyIC-adjuvants for DCs.

References

Anczuków, O., Rosenberg, A. Z., Akerman, M., Das, S., Zhan, L., Karni, R., Muthuswamy, S. K., & Krainer, A. R. (2012). The splicing factor SRSF1 regulates apoptosis and proliferation to promote mammary epithelial cell transformation. *Nature structural & molecular biology*, *19*(2), 220–228. https://doi.org/10.1038/nsmb.2207

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.J., … and Palucka, K. (2000). Immunobiology of Dendritic Cells. Annu. Rev. Immunol., 18, 767-811.

Cao, Y., Bender, I. K., Konstantinidis, A. K., Shin, S. C., Jewell, C. M., Cidlowski, J. A., Schleimer, R. P., & Lu, N. Z. (2013). Glucocorticoid receptor translational isoforms underlie maturational stage-specific glucocorticoid sensitivities of dendritic cells in mice and humans. *Blood*, *121*(9), 1553–1562. https://doi.org/10.1182/blood-2012-05-432336

Chen, M., Huang, L., & Wang, J. (2007). Deficiency of Bim in dendritic cells contributes to overactivation of lymphocytes and autoimmunity. *Blood*, *109*(10), 4360–4367. https://doi.org/10.1182/blood-2006-11-056424

Fuertes Marraco, S. A., Grosjean, F., Duval, A., Rosa, M., Lavanchy, C., Ashok, D., … Acha-Orbea, H. (2012). Novel murine dendritic cell lines: a powerful auxiliary tool for dendritic cell research. Frontiers in immunology, 3, 331. doi:10.3389/fimmu.2012.00331

Fuertes Marraco, S. A., Scott, C. L., Bouillet, P., Ives, A., Masina, S., Vremec, D., … Acha-Orbea, H. (2011). Type I interferon drives dendritic cell apoptosis via multiple BH3-only proteins following activation by PolyIC in vivo. PloS one, 6(6), e20189.

Gruver-Yates, A. L., & Cidlowski, J. A. (2013). Tissue-specific actions of glucocorticoids on apoptosis: a double-edged sword. *Cells*, *2*(2), 202–223. https://doi.org/10.3390/cells2020202

Hafner, A., Corthesy, B., & Merkle, H. (2013). Particulate formulations for the delivery of poly(I:C) as vaccine adjuvant. *Advanced Drug Delivery Reviews*, *65*(10), 1386-1399. https://doi.org/10.1016/j.addr.2013.05.013.

Heidari, N., Miller, A. V., Hicks, M. A., Marking, C. B., & Harada, H. (2012). Glucocorticoid-mediated BIM induction and apoptosis are regulated by Runx2 and c-Jun in leukemia cells. *Cell death & disease*, *3*(7), e349. https://doi.org/10.1038/cddis.2012.89

Kushwah, R., & Hu, J. (2010). Dendritic cell apoptosis: Regulation of tolerance versus immunity. Journal of Immunology, 3, 795-802. doi: 10.4049/jimmunol.1000325.

Marani, M., Tenev, T., Hancock, D., Downward, J., & Lemoine, N. R. (2002). Identification of novel isoforms of the BH3 domain protein Bim which directly activate Bax to trigger apoptosis. Molecular and cellular biology, 22(11), 3577-89.

Murphy, K., & Weaver, C. (2017). Janeway's Immunology (9th ed.). New York, NY: Garland Science.

Sionov, R. V., Vlahopoulos, S. A., & Granot, Z. (2015). Regulation of Bim in Health and Disease. *Oncotarget*, *6*(27), 23058–23134. https://doi.org/10.18632/oncotarget.5492

Trumpfheller, C., Caskey, M., Nchinda, G., Longhi, M. P., Mizenina, O., Huang, Y., Schlesinger, S. J., Colonna, M., & Steinman, R. M. (2008). The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(7), 2574–2579. https://doi.org/10.1073/pnas.0711976105

UniProt (2020). *UniProtKB -O54918 (B2L11_Mouse)*. https://www.uniprot.org/uniprot/O54918