Robustness of ex vivo, human skin as a model of general inflammation

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PURPOSE

For skin-associated, inflammatory diseases such as psoriasis and atopic dermatitis, explant skin has been used to better understand disease pathogenesis and to investigate the capabilities of experimental treatments to alter disease-specific inflammatory pathways. (1-4) Over the past decade, we have worked to combine ex vivo, human skin culture with optimized cytokine cocktails which are designed to mechanistically activate molecular pathways associated with skin inflammation, often linked to Th1, Th2, Th17, or LPS inflammatory mediators. (5-6) Using this activation, drug efficacy for specific targets along those molecular pathways can readily be assessed. However, these pathways and mediators are not limited to diseases of the skin. They have been shown to play significant roles in several types of cancer and inflammatory diseases of the connective tissue such as rheumatoid arthritis and systemic sclerosis. (7-9) In this study, we wanted to determine the utility of our ex vivo skin inflammation models for studies involving general inflammation in diseases beyond the skin. Here we describe the effects of various small molecule inhibitors on molecular targets which are known to be associated with general inflammatory response pathways.

OBJECTIVES

- Harvest and culture healthy, human skin explants and achieve stimulation using our Th1, Th2, Th17, and LPS stimulation cocktails.
- Treat stimulated skin explants basolaterally with various small molecule inhibitors of specific inflammatory pathway targets.
- Run RT-qPCR for each treatment group and compare the gene expression to stimulated samples without treatment to determine the effects of the inhibitors.

METHODS

- *Ex vivo* skin from healthy, human donors was collected and dermatomed to the desired thickness.
- Biopsies were then collected and plated according to our tissue culture setup depicted in Figure 1.
- To mimic systemic delivery, the drug product was diluted in media and added basolaterally to the tissue culture system.
- After plating, the treatment and sample processing steps were as follows:

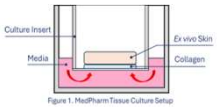
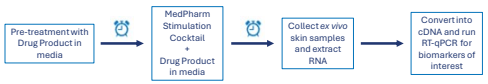


Figure 1. MedPharm Tissue Culture Setup



Molecular Target	Pathway Target	Examples	Product Used
NF-κB	Inflammasome	Bortezomib	Bortezomib
PD-L1	Programmed Cell Death	BMS-936559	BMS-1
HMG-CoA	Statins and Migration	Simvastatin, Pravastatin	Pitavastatin
TNFα	Gen Inflammatory Response	Infliximab, Etanercept	R-7050
IL6R	Gen Cytokine Response	Tocilizumab	LMT-28
CXCR-1/2	Neutrophil Recruitment	Reparixin	MK-7123
CXCR4	Infiltration and Vascular Permeability	Plerixafor	Plerixafor
JAK	Cytokine Cell Signaling	Ruxolitinib	Ruxolitinib
CSF-1R	Macrophage/Monocyte Recruitment	Pexidartinib	PLX-3397
MDSC/RAGE	Pattern Recognition, TLR	Tasquinimod	Tasquinimod

Table 1. Molecular Targets of Interest and their Clinical and Experimental Inhibitors

RESULTS

Figure 2. **Th1 Stimulation**

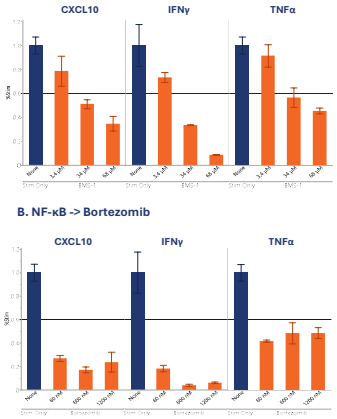


Figure 3. **Th2 Stimulation**

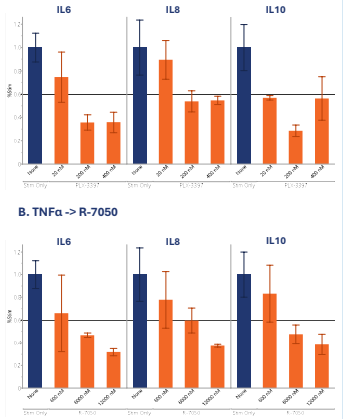
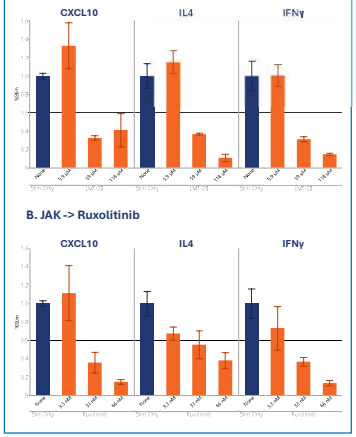


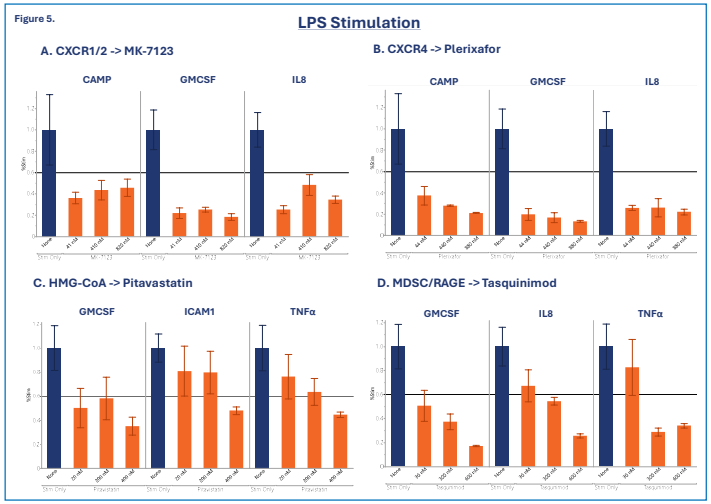
Figure 4. **Th17 Stimulation**



Drug Product	Low	Medium	High
Bortezomib	60 nM	600 nM	1.2 μM
BMS-1	3.4 μM	34 μM	68 μM
Pitavastatin	20 nM	200 nM	400 nM
R-7050	600 nM	6 μM	12 μM
LMT-28	5.9 μM	59 μM	118 μM
MK-7123	41 nM	410 nM	820 nM
Plerixafor	44 nM	440 nM	880 nM
Ruxolitinib	3.3 nM	33 nM	66 nM
PLX-3397	20 nM	200 nM	400 nM
Tasquinimod	30 nM	300 nM	600 nM

Table 2. Concentrations Used for Each Treatment

Figure 5. **LPS Stimulation**



RESULTS (CONT.)

- Healthy, human skin was collected from N = 4 donors (1 per stimulation), dermatomed to 750 ± 100 μm, and biopsies were plated according to MedPharm protocols.
- Treatment groups were composed of n = 4 replicates per concentration (Low, Medium, and High concentrations), each shown in Table 2.
- For each stimulation, a set of biomarkers was chosen for RT-qPCR analysis that encompassed the major inflammatory drivers of the chosen stimulation.
- A subset of these biomarkers for each stimulation and treatment was selected based on two different criteria after normalizing the data to the Stimulation Only treatment group:
  - Presence of a dose response
  - Greater than or equal to a 40% reduction in expression with treatment.
- After analyzing the data, we were able to match each pathway target-inhibitor combination with one of our stimulation regimens which could be used for future drug assessment.

CONCLUSIONS

Molecular Target	Pathway Target	Product Used	Best Model
NF-κB	Inflammasome	Bortezomib	Th1
PD-L1	Programmed Cell Death	BMS-1	Th1
HMG-CoA	Statins & Migration	Pitavastatin	LPS
TNFα	Gen Inflammatory Response	R-7050	Th2
IL6R	Gen Cytokine Response	LMT-28	Th17
CXCR-1/2	Neutrophil Recruitment	MK-7123	LPS
CXCR4	Infiltration & Vascular Perm	Plerixafor	LPS
JAK	Cytokine Cell Signaling	Ruxolitinib	Th17
CSF-1R	Macrophage/Monocyte Recruitment	PLX-3397	Th2
MDSC/RAGE	Pattern Recognition, TLR	Tasquinimod	LPS

Table 3. Molecular Targets of Interest and the Best Stimulation Model to Use

- Using healthy, human skin, we were able to mechanistically stimulate inflammatory responses associated with Th1, Th2, Th17, and LPS-mediated inflammation.
- Each small molecule inhibitor that was examined displayed either a dose-dependent response or maintained a 40% reduction in expression for each inhibitor concentration when compared to the stimulated only group.
- We were able to determine that each pathway target that was investigated could be matched with one of our stimulation models for future experimentation outside of skin inflammatory diseases, expanding their use and robustness into more general inflammation.
- Future experiments will encompass culturing additional donors using the same treatment regimens presented here, testing additional pathway target inhibitors associated with the ubiquitous JAK/STAT, NF-κB, and MAPK pathways, and investigating the effects of anti-inflammatory peptides.

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