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Multianalyte Microphysiometry of Macrophage Responses to Phorbol Myristate Acetate, Lipopolysaccharide, and Lipoarabinomannan

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Abstract

This study examined the hypothesis that mycobacterial antigens generate different metabolic responses in macrophages as compared to gram-negative effectors and macrophage activators. The metabolic activation of macrophages by PMA is a useful tool for studying virulent agents and can be compared to other effectors. While phorbol myristate acetate (PMA) is commonly used to study macrophage activation, the concentration used to create this physiological response varies. The response of RAW-264.7 macrophages is concentration-dependent, where the metabolic response to high concentrations of PMA decreases suggesting deactivation. The gram-negative effector, lipopolysaccharide (LPS), was seen to promote glucose and oxygen production which were used to produce a delayed onset of oxidative burst. Pre-incubation with interferon-γ (IFN-γ) increased the effect on cell metabolism, where the synergistic effects of IFN-γ and LPS immediately initiated oxidative burst. These studies exhibited a stark contrast with lipoarabinomannan (LAM), an antigenic glycolipid component associated with the bacterial genus *Mycobacterium*. The presence of LAM effectively inhibits any metabolic response preventing consumption of glucose and oxygen for the promotion of oxidative burst and to ensure pathogenic proliferation. This study demonstrates for the first time the immediate inhibitory metabolic effects LAM has on macrophages, suggesting implications for future intervention studies with *Mycobacterium tuberculosis*.

Keywords

oxidative burst; gram-negative; mycobacterium; interferon-γ

Introduction

Macrophages provide the primary line of defense for the immune system by combating foreign matter and by inciting reactive oxygen species (ROS) formation, which effectively leads to the cessation of pathogenic proliferation.[1, 2] The formation of ROS begins with the engulfment of the pathogenic agent, which promotes the formation of the NADPH oxidase complex.[2] This complex allows for the reduction of molecular oxygen, which can be further

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converted to superoxide and later hydrogen peroxide and hydroxide, ROSs known to degrade foreign particles during an oxidative burst.[3] To examine these responses, researchers often use a known macrophage activator, phorbol myristate acetate (PMA), to incite macrophage activation and subsequent oxidative burst.[2] Typically PMA rapidly incites the oxidative burst response; however, complete dose responses are unknown and large doses suggest a plateau or oversaturation of the cell, leading to a reduced response.[4] Here, we perform a concentration dependent examination of PMA exposure for an eventual comparison with alternative macrophage effectors. Likewise, agent-specific insight can be enhanced using variations of bacterial membrane virulent components. Understanding these immunological responses to bacterial infection is important to drug development and pathogen eradication.

Lipopolysaccharides (LPS), an endotoxin found in gram-negative bacterial outer membranes, are known to initiate oxidative burst in macrophages solely and synergistically with cytokines such as interferon-γ (IFN-γ)[5] and tumor necrosis factors-α/β.[5-9] Studies have shown that LPS induces oxidative burst in macrophages over the course of hours, however there have been no dynamic experiments performed to observe the metabolic activity of macrophages before, during, and after LPS exposure on the minute time scale.[6-10]

In contrast to LPS from gram-negative bacteria, the interaction of lipoarabinomannan (LAM) from *Mycobacterium tuberculosis* with host macrophages does not lead to the generation of an oxidative burst and instead leaves the bacterium viable and capable of proliferation or dormancy. Tuberculosis is a disease of poverty that has infected millions of people worldwide. The World Health Organization estimates that in 2009 alone, 1.7 million people died from this disease.[11] Tuberculosis resides in the lungs where it interacts with alveolar macrophages and either promotes physical symptoms or remains dormant in granulomas until the host immune system is weakened.[12, 13] Unfortunately, there is no concise understanding of how the macrophage metabolic response to tuberculosis infection differs from the metabolic reaction seen in gram-negative bacterial infection, or other known oxidative stressors.

It remains to be determined how LAM modulates the immune response, whether by inhibiting host defense or by aiding mycobacterial invasion.[14, 15] Three main hypotheses are presented here for the consideration of the intrinsic differences between gram-negative bacteria and mycobacterium with regard to the effects of these antigenic agents on macrophage ROS production. (1) LAM scavenges free radicals allowing pathogenic proliferation.[16, 17] (2) LAM binds to a macrophage surface receptor or otherwise inhibits other signaling molecules to prevent the initiation of oxidative burst.[6, 16, 18, 19] (3) LAM is recognized as antigen by the macrophage and an oxidative response similar to LPS-induced initiation occurs.[6] These hypotheses stem from the inability to assess the dynamics of macrophage metabolism during exposure to LAM. Recently literature utilizes various electrochemical techniques, including the use of microelectrodes and microfluidics, to study oxidative burst.[20-22] This has afforded considerable knowledge regarding cellular production of ROS and reactive nitrogen intermediates (RNI), however these insights typically focus solely on ROS and RNI. In this study, the dynamic metabolic changes of macrophages

exposed to pathogenic agents were measured, increasing the current understanding of macrophage activation.

To study the complex metabolic flux of macrophage activation and oxidative burst, we used the multianalyte microphysiometer (MAMP) to measure extracellular lactate, oxygen, and acidification to increase the knowledge of intracellular effects promoted by LAM exposure. The MAMP provides a novel approach for examining oxidative burst in macrophages by allowing for the real-time collection of data throughout pathogenic agent exposure and recovery. Previous studies from our lab have utilized the MAMP to study an array of biological phenomena including neuronal preconditioning, islets stimulation and 4-hydroxynonenal macrophage activation (unpublished results).[23-28] Here, the MAMP is used to observe the immediate effects of pathogenic agent exposure. LPS exposure was used to determine the changes in cellular respiration that occur from the initiation of oxidative burst. Macrophages that were exposed to LAM could then provide useful metabolic signatures to compare pathways of macrophage activation, oxidative burst, and the capability of pathogenic proliferation.

Materials and Methods

Chemicals and Instrumentation

All materials were used as obtained unless otherwise noted. Alamethicin was purchased from A.G. Scientific, Inc (San Diego, CA) in lyophilized form. A seed of murine macrophages (RAW-264.7, ATCC number **TIB-71**) was obtained through American Type Culture Collection (Manassas, VA) then cultured in our lab using standard cell culture protocol. Glucose oxidase (GOx, Type IIS from *Aspergillus niger*), bovine serum albumin (BSA, fraction V, 96%), glutaraldehyde (glutaric dialdehyde, 25 wt% solution in water), and PMA (stock solution originally in DMSO at 1 mg/1 mL, for molecular biology, ≥99%) were purchased from Sigma. Lactate oxidase (LOx, stabilized) was purchased from Applied Enzyme Technology (Pontypool, UK). Culture media and media supplements were obtained from the Media Core at Vanderbilt University (Nashville, TN) or Mediatech (Manassas, VA). Cytosensor® consumables were purchased from Molecular Devices Corporation (Sunnyvale, CA). For the study of oxidative burst activators, murine IFN-γ was purchased from PeproTech (Rocky Hill, NJ). LPS was purchased commercially through Sigma-Aldrich. Lastly, the following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Purified LAM from *Mycobacterium tuberculosis*, Strain H37Rv, NR-14848.

MAMP Experiments

Experiments were performed using a multianalyte microphysiometer, which couples the Cytosensor® Microphysiometer with a VIIBRE Multi-Channel Potentiostat (Vanderbilt Institute of Integrative Biosystems Research and Education, Nashville, TN). The Cytosensor® Microphysiometer housed the cells in four separate chambers allowing for the collection of extracellular acidification through the light addressable potentiometric sensors (LAPS), as well as maintaining control of temperature and pump speed.[9, 10, 29]

Modified sensor heads included additional electrodes for the simultaneous collection of amperometric currents corresponding to glucose, lactate, and oxygen. Glucose and lactate enzyme films were hand-cast onto the electrodes prior to experimentation to provide for the measureable formation of H_2O_2 (+0.6 V vs. Ag/AgCl; 2 M KCl). A thin coat of Nafion limited biofouling at the oxygen electrode, which was held at a potential of -0.45 V vs. Ag/AgCl (2 M KCl) to reduce molecular oxygen dissolved in solution.

During experimentation the Cytosoft program was set to a flow cycle of 120 s: 80 s of flow followed by 40 s of stop flow. Accumulation of analytes occurred during the stop flow, which improved detection. The perfusion rate of 0.1 mL/min of modified RPMI 1640 (5 mM glucose added) occurred during the 80 s flow period.

Extracellular acidification values were calculated by the Cytosoft program throughout the duration of the experiment.[9, 10] For lactate, and oxygen values, the stop flow current response, i_p, was determined by calculating the difference in current between steady state flow and end of the stop-flow cycle. Calibrations were performed for the lactate sensors, allowing for the determination of analyte produced. Oxygen consumption was determined by calibrating i_p with 0.24 mM, the concentration of dissolved oxygen.[30] The resulting values were normalized against the baseline measurements prior to pathogenic agent exposure.[31] For the PMA studies, the analyzed data for each peak during the 30 min exposure was averaged and used to represent the various concentrations for a dose response-type curve. For all other studies, including LPS and LAM exposures, the percent deviation from the baseline was then plotted against time to indicate the dynamic metabolic response of macrophages to pathogenic agent exposure.

Experimental Protocol

Corning Costar® Transwell cell culture inserts (PTFE, 3 μm pores) housed 2.5×10^5 RAW 264.7 cells (CRL-2278 ATCC). Cells were plated in these inserts 36 h prior to experimentation to allow adequate time for cellular adhesion to the polycarbonate membrane. The incubation occurred at a constant 37 °C with 5% CO_2. Dulbecco's Modified Eagle Medium, 50 mL FBS, 5.0 mL Sodium Pyruvate, 0.5 mL of an antifungal was used as the culture medium, prior to switch to RPMI running media which was used during the experiments. After adherence, a spacer and capsule insert were added to the cell culture insert, allowing for a 3 μL chamber with the addition of the modified sensor head.

PMA exposure was performed using the following concentrations of PMA: 0.0 mM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, and 1 mM. Macrophages were plated ~36 h prior to experimentation and were cultured as mentioned previously, without the addition of any preincubation species. Basal metabolic rates were recorded for one hour prior to the 30 min PMA exposure to ensure adequate baseline equilibration. After exposure, the cells were given an hour recovery time, and then killed to allow for sensor calibrations in the absence of cellular metabolic activity.

For preincubation oxidative burst studies, cells were dosed with 20 ng/mL IFN-γ for 36 h prior to introduction into the MAMP. Basal metabolic rates were recorded for one hour prior to a twenty-minute exposure to 10 μg/mL of LPS, and a 60 min recovery time. For LPS and

LAM exposure studies, the cells were grown under normal culture conditions, without the addition of an oxidative burst activating cytokine. An hour of basal metabolic rates preceded a twenty-minute exposure to 10 μg/mL of LPS or 5 μg/mL of LAM. A subsequent recovery time of one hour was followed by exposure to the alamethicin solution.

After cell death, sensor calibrations were performed. The lactate sensor was calibrated with the following amounts of lactate: 0 mM, 0.05 mM, 0.1 mM, 0.2 mM. After calibrations in the absence of cellular metabolic activity, a baseline was reestablished using standard 5 mM glucose running media.

Results

PMA concentration-dependent activation

Macrophage oxidative burst induction has been continually studied for decades using PMA as an activator.[20, 32-34] This research has afforded the knowledge that protein kinase C (PKC), which leads to NADPH oxidase complex formation and oxidative burst, can be rapidly activated by minute amounts of PMA.[35, 36] However, the actual amount of PMA needed for oxidative burst induction varies wildly in the literature from 2 nM to 20 μM, as does the exposure time from seconds to multiple hours. To better understand the macrophage metabolic response to PMA exposure, we employed the MAMP to (1) examine whether the expected response would plateau or reverse in a concentration dependent manner and to (2) compare the dose response data of known PMA pathways with those of lesser understood agents to see if any mechanistic insight could be gained.

Over the course of a 30 min exposure, PMA responses were averaged and plotted according to the log of their concentrations (Figure 1). Acidification rates slowly increased with increasing concentration up to 100 μM. Lactate production decreased as compared to baseline levels with the lower concentrations of PMA (10 nM to 1 μM) and then the dose response inverted and lactate production increased when exposed to higher concentrations of PMA (10 μM to 100 μM). Interestingly, the oxygen dose response hit a plateau between 100 nM and 1 μM, and then steadily decreased with increasing concentrations of PMA (10 μM to 100 μM). A higher dose of 1 mM was also tested and during this exposure, the change in baseline of all analytes trended back toward 0% change in baseline. This suggests an oversaturation or desensitization toward PMA exposure at this higher concentration.

This data supports the desensitization of macrophages to activators such as PMA as previously reported by Berton and Gordon.[4] They reported lasting desensitization of macrophages to a second PMA exposure after an initial low concentration exposure, as well as an oversaturation of the ligand recognition system responsible for inciting oxidative burst.[4] Our data demonstrates the metabolic inhibitory effect of oversaturation by PMA at higher dosages. Lactate production, acidification rates, and oxygen consumption plateau and decrease at higher concentrations of PMA exposure, exhibiting a consistent metabolic decrease due to lack of available recognition cites to promote oxidative burst. In addition to expanding on the previously reported macrophage desensitization by PMA exposure, these dose response curves enable a pathway comparison with alternative methods of macrophage oxidative burst inducers.

Lipopolysaccharide macrophage activation

LPS is a virulent factor found in the outer membrane of gram-negative bacteria. Along with PMA, LPS is often used to activate and incite oxidative burst responses in macrophages. To examine the differences between the metabolic responses of macrophages to PMA, LPS, and LAM we first looked at the metabolic relationship between LPS exposure alone and LPS exposure after cytokine priming of macrophages. Beginning by examining macrophage metabolic change within the first few minutes of exposure to LPS, a metabolic signature of ROS production was established. These experiments, 10 μg/mL LPS exposure,[5, 37] indicated a lasting increase in lactate production and extracellular acidification, while also showing an increase in oxygen consumption during exposure that did not last throughout recovery (Figure 2, Table 1). The metabolic response of macrophages to LPS indicates oxidative burst initiation in addition to recruiting analytes to promote transcription.

Synergistic activation with LPS and IFN-γ

To better understand how this metabolic response correlates to the reported[37] synergistic effects of cytokines and LPS macrophage activation, priming of cells with IFN-γ was undertaken. During exposure, primed cells (20 ng/mL IFN-γ for 36 hrs, then 10 μg/mL LPS for 20 minutes) exhibited a rapid, marked increase in oxygen consumption, indicating oxidative burst initiation (Figure 3, Table 1). However, lactate production and extracellular acidification drastically decreased, similar to reports by Costa Rosa et al. on the effects of adrenaline exposure to macrophages.[38] Our data suggests that all consumed glucose energy is being used by the NADPH oxidase complex to allow for ROS production, highlighting the decreases seen by alternate macrophage activators.[39]

Macrophage exposure to lipoarabinomannan

Tuberculosis can remain dormant for extended periods of time, without any type of symptom presentation, which greatly contrasts the clinical presentations of gram-negative bacterial infections. In order to understand how LAM acts differently than LPS, with and without cytokine priming, we observed the metabolic response of macrophages with a 5 μg/mL LAM exposure (Figure 4, Table 1). During LAM exposure there is no remarkable change of cellular metabolism, which differs greatly from the LPS exposure studies. Also different from LPS, upon removal of LAM from the chamber, there were statistically significant changes in peak heights. The most striking difference in these metabolic profiles is the decrease of oxygen consumption after removal of LAM. This indicates that LAM does not activate macrophages in the same ways as LPS or IFN-γ. Instead, the sharp decline following exposure indicates that LAM either does not initiate oxidative burst or prohibits it by interfering with ROS production, and this eventually causes cessation of normal metabolic activity. A similar deactivation of macrophage oxidative burst phenomenon was reported in the literature during exposure to *Histoplasma capsulatum*, in which case the findings suggested a structural component to the bacteria was the interfering factor.[40]

With this previous literature suggesting structural components being the cause deactivation, we further investigated a structural component of LAM. LAM is composed of a mannosyl-phosphatidyl-*myo*-inositol anchor, a polysaccharide backbone, and a mannose capping motif.[41, 42] From the mannose core, there can be variations in arabinofuranosyl side chains,

leading to variation in virulence.[41] In order to examine the effect that mannose can have on macrophages, since it is through the mannose receptors that LAM is known to bind, the metabolic response during mannose exposure was examined (Figure 5, Table 1).[18] There was a statistically significant increase in extracellular acidification and oxygen consumption, along with a decrease in lactate production. The increase in oxygen consumption indicates that aerobic respiration is increasing, which is expected upon exposure to excess sugar. Furthermore, lactate production decreased, which was not seen during or following LAM exposure. The presence of a marked metabolic change during mannose exposure as well as an increase in oxygen consumption indicates that the mannose cap of LAM alone was not responsible for the metabolic change in macrophage respiration.

Discussion

PMA usage by multiple research teams has led to the valuable insight that PMA is an effective macrophage activator and oxidative burst inducer.[20, 32-34] Our data depicts the importance of PMA as an oxidative burst activator at low concentration dosages, and also agrees with previous findings that PMA can deactivate macrophages upon overstimulation.[4] The dose responses reported here provide further metabolic insight into the PMA activation pathway,[35, 36] as well as the macrophage metabolic response to desensitization by PMA overexposure. In addition to increasing the understanding of the metabolic effects by PMA, this data is useful for our metabolic comparisons with clinically relevant virulence factors such as LPS and LAM.

Both PMA and LPS were found to act rapidly to induce oxidative burst. However, our metabolic profile of lactate production during LPS exposure suggests that there is recruitment of glucose to promote alternative methods of macrophage response in addition to ROS production. LPS has been shown to activate toll-like receptor 4, which in turn activates nuclear factor kappa B, leading to the induction of iNOS.[43] Production of RNI can take hours, whereas the production of ROS occurs rapidly. The increase in metabolic response that we observed is likely to be the metabolic signature of LPS induced oxidative burst. LPS activation of macrophages toward oxidative burst is thought to occur over the course of hours and is often reported as being heavily influenced by synergistic cytokines.[6-10] The 20 min exposure may not be long enough to observe all antimicrobial defenses mediated by LPS, however the increase in metabolic activity suggests that the cells recognize LPS as pathogenic and begin to funnel glucose and oxygen into the promotion of cytokine production to influence oxidative burst.

Interestingly, we found that IFN-γ allows oxidative burst to occur immediately upon LPS exposure, with an instant decrease in lactate and acidification. This differs greatly from PMA and LPS (with no pre-incubation) activation, where PMA (at high, but not oversaturated, concentrations) and LPS increased both lactate production and extracellular acidification rates. IFN-γ interacts with the plasma membrane of the macrophage by binding to Janus kinases. [8, 44] This binding allows for tyrosine phosphorylation of cytoplasmic signal transducer and activator of transcription (STAT). STAT monomers will dimerize and enter the nucleus of the cell, binding to gamma activation sites, eventually leading to activation of IFN-γ gene transcription.[8, 44] Thus, when macrophages are primed for 36 h with IFN-γ, a

signaling cascade allows for continual production of IFN-γ and sensitizes the cell for an LPS assault. Upon exposure, the immediate synergistic effects of IFN-γ and LPS are seen and the cell efficiently undergoes oxidative burst instead of having to promote alternative cellular methods of recruitment for ROS production.

LAM has been extensively studied and is known to inhibit macrophage IFN-γ activation and response.[6, 7] Additional studies have proven that LAM integrates into the macrophage plasma membrane, thus disturbing cytokine signaling, calcium flux, and cellular proliferation.[45] Our data parallels these findings, as there was no remarkable metabolic response during LAM exposure. The delay in response is potentially due to a cellular paralysis, or macrophage deactivation, initiated by LAM, enabling the continuation of normal metabolic flux. Instead of allowing activation of macrophages to incite oxidative burst, macrophages do not obtain instruction to activate the NADPH oxidase complex and thus never create ROS to combat LAM exposure. Therefore, we see little change in metabolic profiles during exposure to LAM. Upon removal of LAM, there was an increase in lactate production and extracellular acidification, similar to the response seen during exposure to LPS. Instead of an expected increase in oxygen consumption that we observed with LPS exposure, we observe a decrease after removal of LAM, indicating a lack of oxidative burst. It seems that the presence of LAM effectively inhibits any metabolic response to ensure that there is no consumption of glucose or oxygen to promote oxidative burst, therefore ensuring pathogenic proliferation. Interestingly, a recent study indicates that the dramatic variation in the induction of inflammatory responses are linked to genetic diversity between strains and that the strains responsible for a low inflammatory response were subsequently more virulent.[46] The lack of metabolic activity during LAM exposure followed by statistically significant decreases in oxygen consumption during recovery illustrates the high virulence associated with an initial low inflammatory response.

Understanding the modes of action for pathogenic species opens up the opportunity for specifically tailoring pharmaceutical development. In the present study, we showed that our MAMP was successful in measuring the real-time, dynamic metabolic flux of macrophages undergoing pathogenic invasion. We successfully measured the delayed initiation of oxidative burst by LPS exposure, as well as the immediate oxidative burst initiation by IFN-γ primed cells. We then utilized this information to compare the macrophage metabolic response to LAM, a pathogenic species that has conflicting modes of action. This data suggests that LAM does not initiate oxidative burst, but more likely interferes with normal cellular signaling, prompting cellular paralysis and reducing oxygen consumption. Future work should focus on the lack of oxidative burst incited by LAM exposure and the signaling pathways inhibited by LAM.

Acknowledgments

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Abbreviations

BSA	bovine serum albumin
GOx	glucose oxidase
IFN-γ	interferon-γ
LOx	Lactate oxidase
LAPS	light addressable potentiometric sensors
LAM	lipoarabinomannan
LPS	lipopolysaccharide
MAMP	multianalyte microphysiometer
PKC	protein kinase C
RNI	reactive nitrogen intermediates
ROS	reactive oxygen species
STAT	signal transducer and activator of transcription
PMA	phorbol myristate acetate

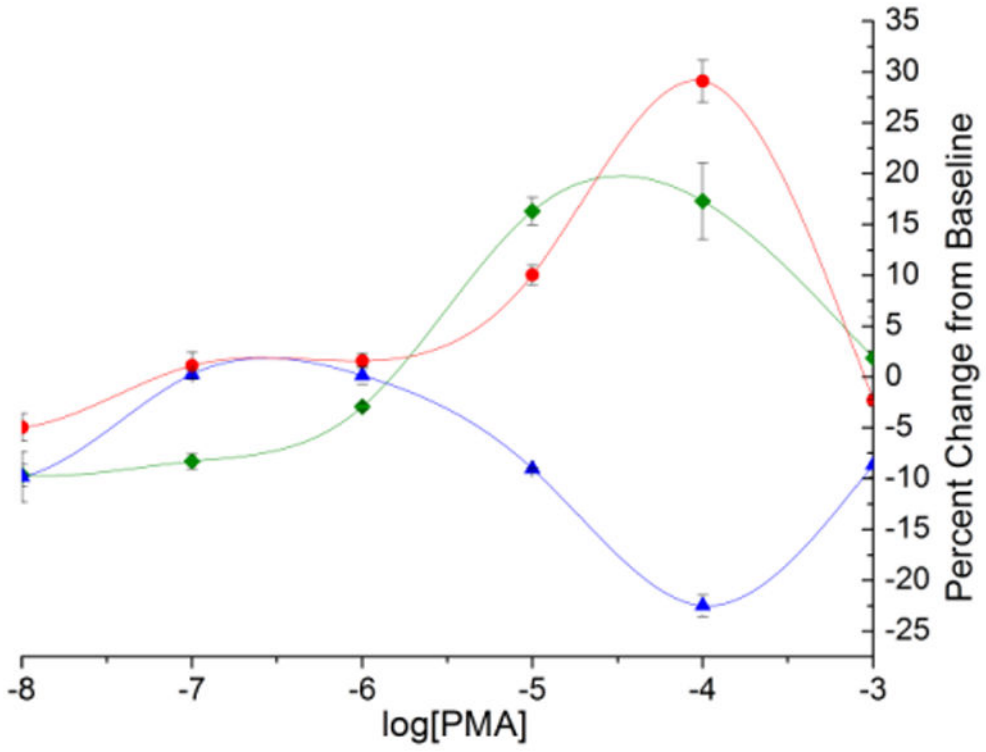

Figure 1.

Averages of thirty minute PMA exposures at increasing concentrations. Concentrations represented include 10 nM (-8), 100 nM (-7), 1 μM (-6), 10 μM (-5), 100 μM (-4), and 1 mM (-3). Lactate production (green), oxygen consumption (blue), and acidification rate (red) dose responses are shown. Data shows initial decreases in metabolic response, followed by increases in response, and culminates in a return to baseline which indicates a desensitization of macrophages to PMA exposure.

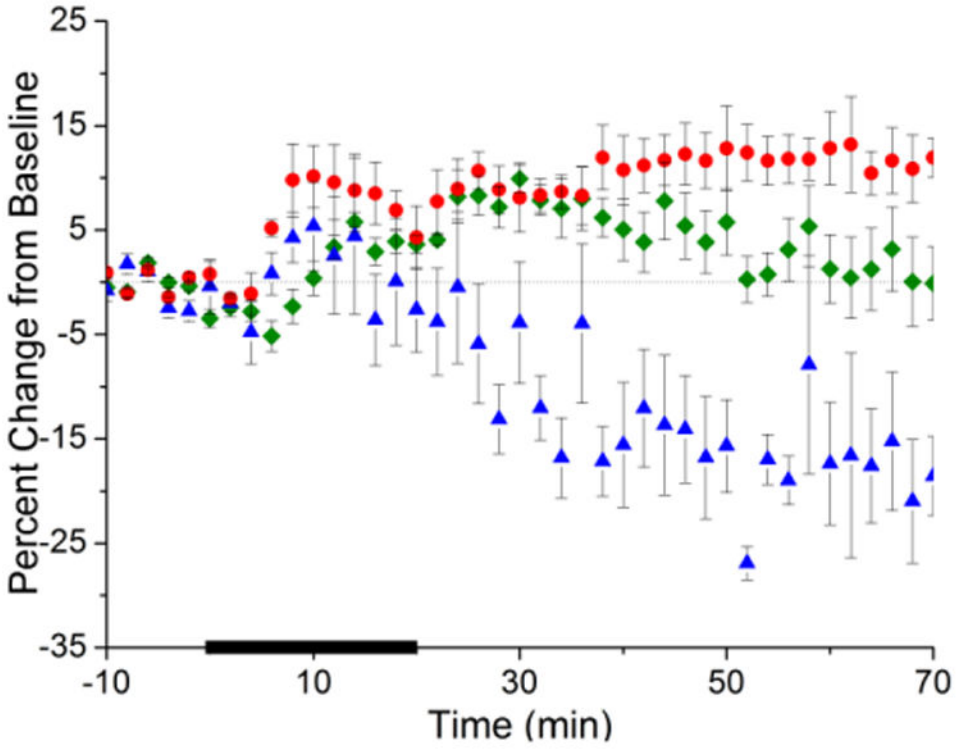

Figure 2.

Twenty minute 10 μg/mL LPS exposure (indicated by black bar). The dashed line indicates a zero percent change from baseline. Lactate (green diamond, n=6, ± 0.2%) shows a peak height increase of 5.8% ± 1.0%, suggesting energy recruitment for eventual iNOS transcription. Oxygen (blue triangle, n=3, ± 4.1%) shows a peak height increase of 6.0% ± 4.3%, indicative of oxidative burst initiation. Acidification (red circle, n=3, ± 1.7%) also increases, 10.2% ± 3.0%, again showing energy recruitment. Additionally, there was a clear response seen during the exposure time, indicating that LPS exposure response occurs relatively rapidly and is sustained for two of the analytes.

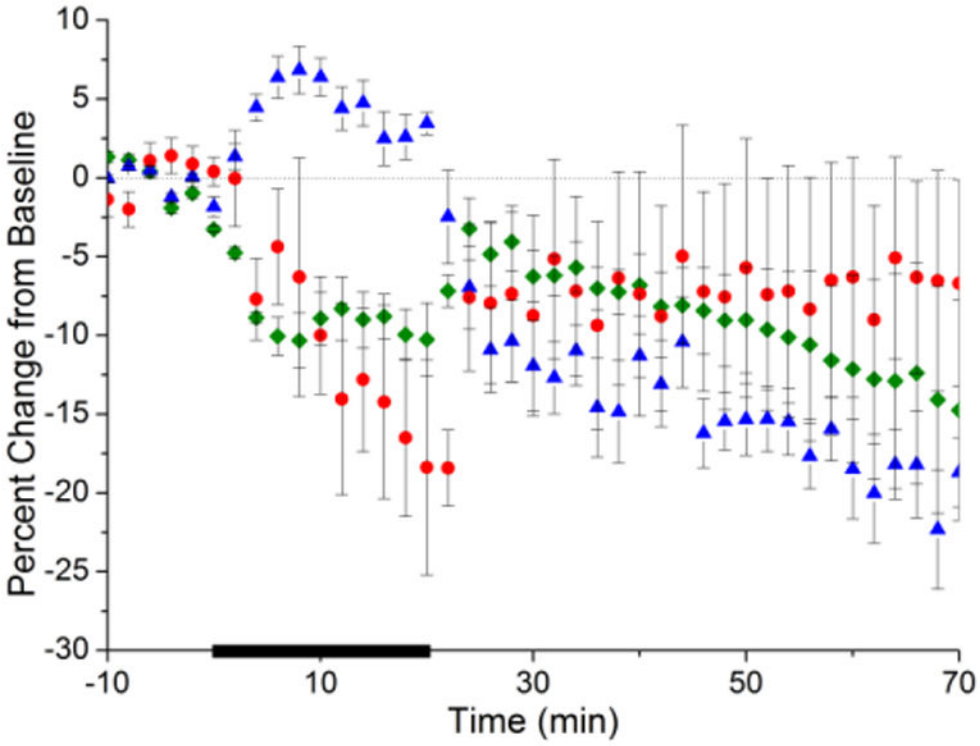

Figure 3.

IFN-γ primed cells (20 ng/mL) with a twenty-minute 10 μg/mL LPS exposure (indicated by black bar). The dashed line indicates a zero percent change from baseline. Here, lactate (green diamond, n=3, ± 0.4%) shows an immediate peak height decrease of 10.3% ± 1.7%, suggesting all available energies are being utilized to promote oxidative burst. Oxygen (blue triangle, n=3, ± 0.8%) shows a peak height increase of 6.8% ± 1.5%, indicative of oxidative burst initiation. Acidification (red circle, n=3, ± 5.0%) also decreases instantly. The response seen here was immediate and indicated that IFN-γ primed the cells for oxidative burst initiation prior to LPS exposure.

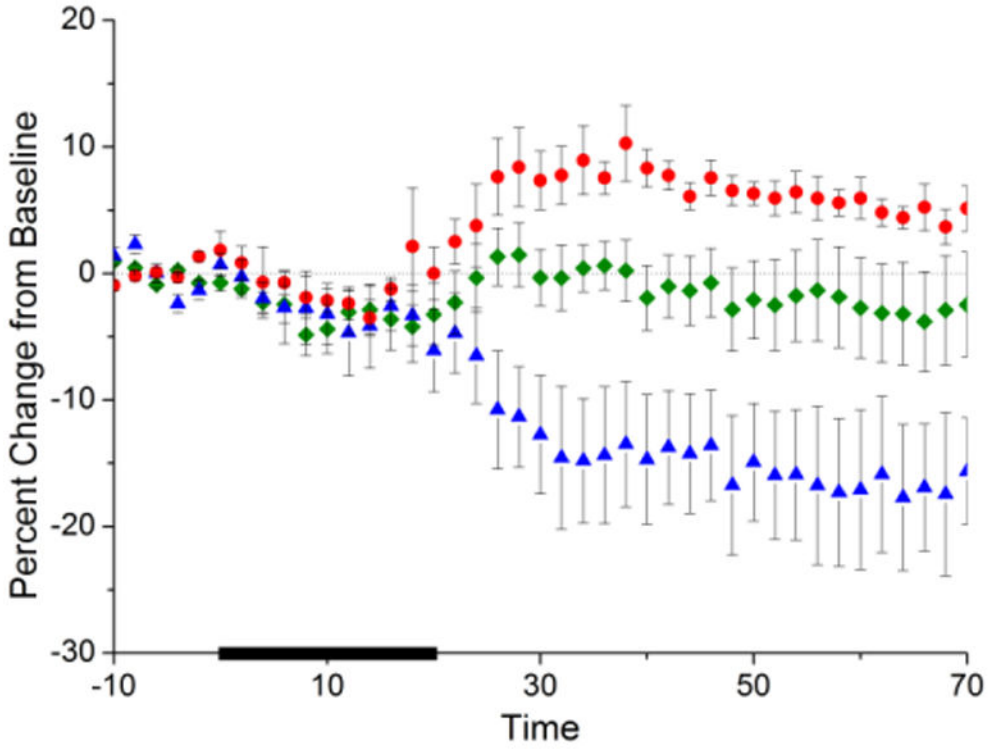

Figure 4.

Twenty minute, 5 μg/mL LAM exposure (indicated by black bar). The dashed line indicates a zero percent change from baseline. Lactate (green diamond, n=4, ± 0.9%), oxygen (blue triangle, n=3, ± 2.6%), and acidification (red circle, n=5, ± 2.0%) show no remarkable metabolic change during exposure. Upon removal of LAM, lactate production and extracellular acidification increase, while oxygen consumption decreases. This highlights the ability of LAM to prevent metabolic changes during exposure, and to inhibit oxidative burst initiation after exposure.

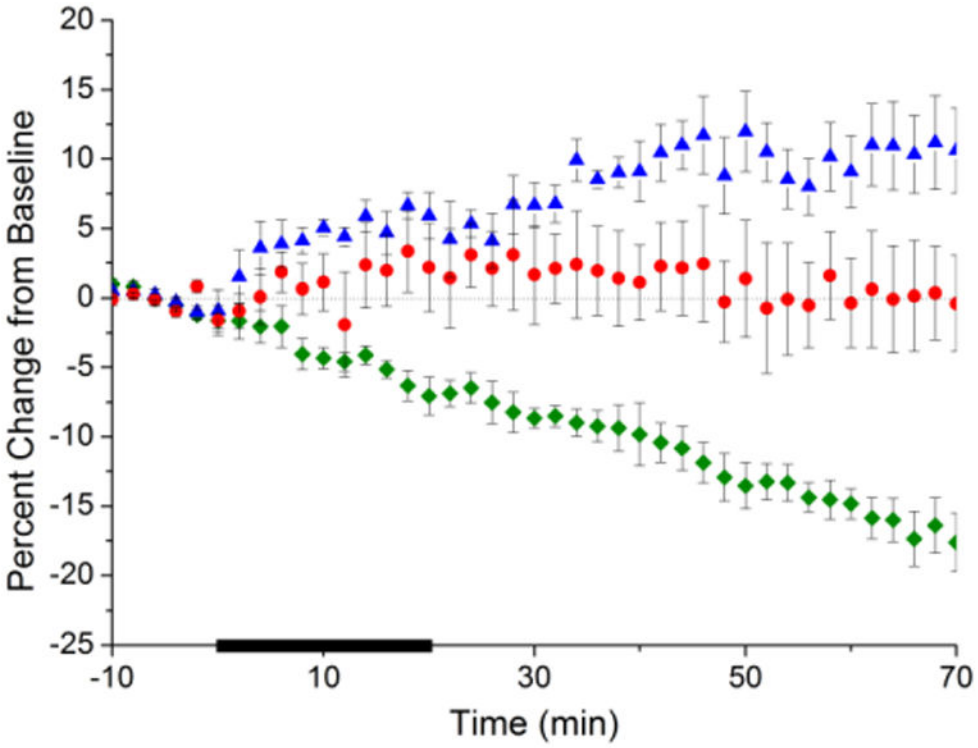

Figure 5.

Twenty minute 27.4 ng/mL mannose exposure (indicated by black bar). The dashed line indicates a zero percent change from baseline. Lactate (green diamond, n=4, ± 1.6%) shows a change of -7.1 ± 1.4% which was not seen upon exposure to LAM. Oxygen (blue triangle, n=4, ± 2.1%) shows an increase of 6.6 ± 1.0% indicating an increase in aerobic respiration upon exposure to excess sugar. Acidification (red circle, n=4, ± 2.1%) also increases, 3.4 ± 2.9%. These changes indicate that the mannose cap of LAM was not solely responsible for the change in macrophage metabolism.

Table 1

Macrophage metabolic responses toward bacterial component exposures. The mean peak height change and the standard errors are shown for each analyte.

	Lactate	Oxygen	Acidification
LPS	5.8 ± 1.0% *	6.0 ± 4.3% *	10.2 ± 3.0% *
IFN-γ primed LPS	-10.3± 1.7% *	6.8% ± 1.5% *	-18.4 ± 6.8%*
LAM (During exposure)	-0.7 ± 0.6%	0.7 ± 0.9%	10.3 ± 4.6% *
LAM (After exposure)	1.5 ± 2.5%	-17.7 ± 5.8% *	10.3 ± 3.0% *
Mannose	-7.1 ± 1.4% *	6.6 ± 1.0% *	3.4 ± 2.9%

* Asterisk (*) notation indicates $p<0.05$ for the mean basal metabolic rates versus bacterial component exposure.