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Beryllium Alters Lipopolysaccharide-Mediated Intracellular Phosphorylation and Cytokine Release in Human Peripheral Blood Mononuclear Cells

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Abstract

Beryllium exposure in susceptible individuals leads to the development of chronic beryllium disease, a lung disorder marked by release of inflammatory cytokine and granuloma formation. We have previously reported that beryllium induces an immune response even in blood mononuclear cells from healthy individuals. In this study, we investigate the effects of beryllium on lipopolysaccharide - mediated cytokine release in blood mononuclear and dendritic cells from healthy individuals. We find that *in vitro* treatment of beryllium sulfate inhibits the secretion of lipopolysaccharide-mediated interleukin 10, while the release of interleukin 1 β is enhanced. Additionally, not all lipopolysaccharide - mediated responses are altered, as interleukin 6 release is unaffected upon beryllium treatment. Beryllium sulfate treated cells show altered phosphotyrosine levels upon lipopolysaccharide stimulation. Significantly, beryllium inhibits the phosphorylation of signal transducer and activator of transducer 3, induced by lipopolysaccharide. Finally, inhibitors of phosphoinositide-3 kinase mimic the effects of beryllium in inhibition of interleukin 10 release, while they have no effect on interleukin 1 β secretion. This study strongly suggests that prior exposures to beryllium could alter host immune responses to bacterial infections in healthy individuals, by altering intracellular signaling.

Keywords

Beryllium; Cytokines; Human; Lipopolysaccharide

INTRODUCTION

Chronic beryllium disease (CBD) is a granulomatous lung disease caused by beryllium exposure in susceptible individuals.⁽¹⁾ 2–16% of exposed individuals develop disease,⁽²⁾ and susceptibility has been associated with HLA-DP alleles possessing a glutamic acid at position 69 (Glu69) of the β chain.⁽³⁾ Approximately 15% of CBD patients, however, do not possess Glu69 containing HLA-DP alleles, suggesting the importance of other factors, genetic as well as environmental, in development of disease.⁽⁴⁾

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Endotoxin or lipopolysaccharide (LPS) is a structural component of membranes of gram-negative bacteria and a potent inflammatory agent. Exposure to endotoxin is epidemiologically related to inflammatory airway diseases,⁽⁵⁾ and can also exacerbate reactive airway disease in asthmatics.⁽⁶⁾ Experimentally, a single exposure of aerosolized LPS has been reported to be sufficient to induce airflow obstruction within minutes and persist for up to 48 hours.⁽⁷⁾ Additionally, inhaled LPS can induce the release of proinflammatory cytokines such as interleukin (IL)1 β , tumor necrosis factor (TNF) α and IL6.⁽⁸⁾

Toll-like receptor (TLR) 4 mediates the innate immune responses to LPS and polymorphisms in the receptor are associated with a blunted response to endotoxin *in vitro*, and a diminished airway obstruction after inhaled endotoxin.⁽⁹⁾ TLRs and innate immunity have also been implicated in host responses to atmospheric pollutants. TLR2 mediates airway epithelial cell responses to air pollution particles,⁽¹⁰⁾ and TLR4 is reported to be important in the inflammatory response to residual oil fly ash (ROFA).⁽¹¹⁾ Components of ROFA, specifically transition metals, are suggested to play a prominent role in the net pulmonary response.⁽¹²⁾ While some of these effects are mediated by contaminating particle-associated microbial matter,⁽¹⁰⁾ it is speculated that some may be secondary effects of upregulation of endogenous TLR ligands, after oxidant-induced airway injury.⁽¹¹⁾ Finally, microbial stimulation, by *Mycoplasma fermentans* and its cognate lipopeptide, can modulate the cellular responses induced by ROFA, in synergistically stimulating IL-6 release,⁽¹³⁾ suggesting complex interplay between particulate and microbial environmental factors.

The effects of environmental factors, such as the presence of low levels of endotoxin, on host response to beryllium, or the effects of prior exposures to beryllium on the host innate immune response have not been examined previously. Significantly, adjuvant effects of beryllium in mice and rabbits have been noted previously.⁽¹⁴⁾ We hypothesized that beryllium exposure may alter the innate immune response to bacterial components such as LPS. In this study, we examined the host response to LPS in immune cells exposed to beryllium *in vitro*. We find that beryllium treated cells exhibit altered cytokine release and intracellular phosphorylation profiles in response to LPS. Results presented here suggest, for the first time, that individuals exposed to beryllium may have altered innate immune responses to bacterial infections.

MATERIALS AND METHODS

Cells and reagents

All protocols for handling human blood cells and beryllium samples were previously approved by the Central Beryllium Institutional Review Board and by the LANL Institutional Biosafety Committee. Poietics® peripheral blood mononuclear cells (PBMCs) and donor matched dendritic cells (DCs) from healthy donors were obtained from Lonza (Walkersville, MD) and were grown in lymphocyte growth medium-3 (LGM™-3) (Lonza, Walkersville, MD). Healthy donors are characterized as individuals having no prior reported exposure to beryllium and lung or infectious disease. Endotoxin was removed from the beryllium and aluminum stock solutions using EndoTrap® endotoxin removal systems (Lonza Walkersville, MD). Cell viability during reconstitution of the cells was tested by LIVE/DEAD® cell kit for mammalian cells (Invitrogen, Carlsbad, CA). Purified, biotinylated antibodies for sandwich enzyme-linked immunosorbent assay (ELISA) and streptavidin horseradish peroxidase (HRP) were obtained from PharMingen (San Diego, CA). Ultrapure *E. coli* LPS 011:B4 was obtained from InvivoGen (San Diego, CA) and has been previously been employed in our laboratory to study TLR4 signaling.⁽¹⁵⁾ This LPS is extracted by successive enzymatic hydrolysis steps and purified by a phenol-triethylamine-deoxycholate extraction protocol by the manufacturer. LPS was suspended in water,

vortexed and aliquots of the solution were sonicated for 10 min prior to each use. Phosphoinositide-3 kinase (PI3K) inhibitors, wortmannin and LY294002 were purchased from Cell Signaling Technology (Danvers, MA). Anti-phosphotyrosine antibody against signal transducer and activator of transcription 3 (STAT3) phosphotyrosine 705 (α pSTAT3); antiphosphotyrosine (α pTyr, PY99) and anti-actin (α actin) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture and treatment

The effects of beryllium sulfate (BeSO_4) treatment on cytokine release were studied using an *in vitro* cell model containing PBMC and donor matched DC in a physiologically relevant ratio 20:1. This cell model has previously been used by us and others to study beryllium-mediated cytokine and chemokine release from healthy individuals. ⁽¹⁶⁾

PBMCs (5×10^7 cells) and DC (2.5×10^6 cells) were thawed out and washed according to manufacturer's protocols. PBMC and DC's were mixed (20:1) and incubated at a concentration of 1×10^6 cells/mL at 37 °C in a humidified environment of 5% CO_2 . Cells were treated with 100 μM metal sulfate alone or pre-, co- or post-treated with 1 $\mu\text{g/mL}$ LPS as indicated in experiments. Cells were spun down at 1000g for 10 min allowing the separation of supernatant from cells, and the cell free-supernatants were subjected to ELISA analyses. Aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$) was used as a control metal for evaluating BeSO_4 specific effects, as it is similar to BeSO_4 in its chemical properties. For pharmacological inhibitor treatments, cells will subjected to 5 nM wortmannin or 5 μM LY294002 for 5 hours prior to LPS/metal sulfate treatments.

Cytokine measurements

Conditioned media were collected from cells treated under indicated conditions and the cytokine released into the cell supernatant was measured by using commercial sandwich ELISA (PharMingen, San Diego, CA), using manufacturer recommended protocols. Routine ELISA's were performed for cytokines IL10, IL6, IL8, IL1 β , IL12p40 and TNF α . Quantitation of the released cytokine was accomplished by normalization of the ELISA data with a standard cytokine dose curve. In all experiments, measurements were done in triplicate and number of samples (n) included in every assay is 3–5 unless otherwise indicated.

Protein analyses

Cell pellets were washed once in cold phosphate buffered saline (PBS) and then lysed using a modified RIPA buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mg/mL aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4 and 1 mM NaF). The lysates were spun down at 15,000g for 15 min after 10 min incubation with lysis buffer on ice. Protein concentration was determined using the Bio-Rad protein kit. Samples were denatured by boiling with Laemmli sample buffer (Bio-Rad, Hercules, CA) and subjected to gel electrophoresis. Proteins separated on gels were transferred onto immobilon membranes, and probed by Western blotting.

Western blotting

Membranes were blocked in bovine serum albumin (BSA) solution (5% BSA w/v in PBS-0.5% Tween 20 (PBST)) overnight and then incubated with 1:5000 of the α pTyr, or the α pSTAT3 antibodies (5% BSA in PBST) for 1h at RT. Blots were washed three times in PBST followed by incubation for 1h at RT in 1:50,000 HRP-conjugated secondary antibody (5% BSA in PBST). Finally blots were washed five times in PBST, incubated with substrate (Pierce Supersignal West Dura Extended Duration Substrate, Pierce, Rockford, IL) and

chemiluminescence was quantitatively measured using the Molecular Imager Chemidoc XRS System (Bio-Rad, Hercules, CA).

Statistical analysis

One-way ANOVA and Mann-Whitney tests were used to determine significance between various treatments. Data are expressed as mean (M) $\pm$ SD (n=3–5). All statistical analyses were performed using Sigma Stat 3.5 software. A p value of <0.05 was considered statistically significant.

RESULTS

Beryllium sulfate inhibits LPS- mediated IL10 release

Co-cultures of PBMC's and donor matched DCs were evaluated for cytokine release profiles in response to LPS and metal sulfate treatment. LPS stimulation triggers induction in IL10 release at 24 hours (Figure 1A). Pre-treatment of cells with BeSO₄ followed by LPS treatment (Figure 1B), led to inhibited LPS-mediated IL10 release (39.84 $\pm$ 19.3%) compared to no-metal treated control cells. Co-treatment of cells with BeSO₄ and LPS had a similar effect on inhibition of IL10 release (57.05 $\pm$ 14.75%) as BeSO₄ pre-treatment. Significantly, this effect was not observed when cells were pre-treated with LPS prior to BeSO₄ treatment. These data very strongly suggest that prior cellular exposure to beryllium has the ability to inhibit LPS-mediated IL10 release in immune cells from healthy individuals.

BeSO₄ induces LPS-mediated IL1 β release

Monocytes and macrophages produce inflammatory cytokine IL1 β , in acute infection, by recognition of LPS by TLR4. ⁽¹⁷⁾ LPS induces IL1 β release in our mixed cell model (Figure 1A, p <0.05). We determined the effect of beryllium on IL1 β release in cells from different donors (Figure 1C). The LPS-stimulated IL1 β release in all donors showed a beryllium dependency. Beryllium pre-treatment of cells induced the LPS-mediated IL1 β release by 48.5 $\pm$ 18.8%. Pre-treatment of LPS followed by metal showed no effect of beryllium. Finally, co-treatment of beryllium and LPS induced IL1 β by 200 $\pm$ 81.2%. Thus beryllium pre-treatment and co-treatment enhances the IL1 β release induced upon LPS stimulation of these cells.

BeSO₄ has no effect on LPS-mediated IL6 release

The ability of BeSO₄ to inhibit IL10 and induce IL1 β release from cells in response to LPS indicates that either beryllium has the ability to sequester LPS, thereby preventing all LPS responses, or to partially modulate a subset of LPS-mediated cellular responses. In order to determine if the presence of beryllium somehow prevents LPS recognition by sequestration or an alternate unknown mechanism, we examined release of other cytokines from these cells. Consistent with our previous report that BeSO₄ inhibits IL6 release in cells from healthy individuals, ⁽¹⁶⁾ IL6 release was inhibited in beryllium treated cells (40% $\pm$ 21.1%) (Figure 1D). IL6 release was stimulated upon LPS treatment (Figure 1A) and the LPS induced IL6 release was not altered by pre-, co- or by post-treatment with BeSO₄. This suggests that beryllium does not sequester LPS binding or the cognate activation of these cells. The inhibitory effects of BeSO₄ on LPS-mediated IL10 release, and the inductive effects on LPS-mediated IL1 β release are not due to blocking or the sequestration of LPS from these cells, since the IL6 release from these cells is similar to LPS control-treated cells. It thus seems likely that beryllium is somehow able to alter only a subset of the cellular activation pathways induced upon LPS engagement.

In dendritic cells, BeSO₄ inhibits IL10 and induces IL1 β release

Next we examined the effect of beryllium on IL10 and IL1 β release using only the antigen presenting DCs in the absence of responder T cells (present in PBMCs). While LPS did not induce IL8 or IL12p40 release in beryllium treated DC, there was significant induction of TNF α and IL6 release upon LPS stimulation (Figure 2). Furthermore, BeSO₄ inhibited IL10 release in LPS-stimulated DC by ~ 38 %, and it stimulated IL1 β release by ~ 13%, suggesting that T cells and other cell types might also contribute to the enhanced effects in IL10 and IL1 β seen in experiments using mixed cell models (Figure 1). These data also suggest that beryllium can partially modulate the cellular responses mediated by LPS on antigen presenting cells. It is notable that while LPS does not induce IL1 β and IL10 significantly in DCs (~ 6 and 10% respectively), the effect of beryllium is observed only in LPS-stimulated cells, suggesting that cellular activation mediated by LPS, although does not appear to have a direct effect on IL1 β /IL10 release in these cells, may indirectly impact their regulation.

Tyrosine phosphorylation mediated intracellular signaling is altered in cells treated with BeSO₄

Myeloid cells stimulated with LPS are known to show enhanced tyrosine kinase activity.⁽¹⁸⁾ In order to directly examine effects of metal pre-treatment on cellular signaling in PBMC:DC mixes, we examined total phosphotyrosine levels in whole cell lysates from cells stimulated with LPS in the presence of either Al₂(SO₄)₃ or BeSO₄ (Figure 3A). Interestingly, while control-LPS and Al₂(SO₄)₃-LPS treated cells showed a similar phosphotyrosine pattern in whole cell lysates, BeSO₄-LPS treated cells showed an inhibition of phosphotyrosine of at least one protein band of molecular weight ~ 82 kDa. This suggests that beryllium treatment could alter tyrosine phosphorylation mediated intracellular signaling cascades induced upon LPS stimulation in these cells.

Signal transducer and activator of transcription 3 (STAT3) is a dominant mediator of the anti-inflammatory effects of IL10 in human macrophages,^(19, 20) and anti-inflammatory response is thought to be a STAT3-dependent, generic cytokine signaling pathway.⁽²¹⁾ STAT3 requires tyrosine phosphorylation for maximal DNA binding activity,^(22, 23) and LPS stimulation of macrophages substantially enhances STAT3 phosphorylation.⁽²⁰⁾ We examined tyrosine phosphorylation of STAT3 in BeSO₄ pre-treated cells stimulated with LPS. STAT3 is inducibly tyrosine phosphorylated upon LPS engagement (Figure 3B). While Al₂(SO₄)₃ pre-treatment had no effect on STAT3 phosphotyrosine, BeSO₄ inhibited STAT3 phosphorylation by ~ 35% (Figure 3B, C).

Inhibitors of PI3K inhibit LPS mediated IL10 release but have no effect on IL1 β or IL6

PI3K activation in response to LPS has previously been noted in various cell types^(24, 25) and it plays a role in IL10 release from monocytes stimulated with *Porphyromonas gingivalis* LPS,⁽²⁶⁾ and regulation of IL1 β secretion in human monocytes.⁽²⁷⁾ Consistently, upon pre-treatment of cells with two pharmacological inhibitors of PI3K, wortmannin⁽²⁸⁾ and LY294002,⁽²⁹⁾ the LPS-mediated IL10 release was inhibited by 66% and 71% respectively (Figure 4). This inhibition is comparable to the effects of beryllium on the LPS-mediated IL10 release in these cells (~ 40 % inhibition, Figure 1). Inhibition of PI3K in these cells had no effect, whatsoever, on the release of cytokines IL1 β and IL6, suggesting PI3K-independent mechanisms for their release. Thus, cells treated with beryllium mimic IL10 release responses of cells treated with inhibitors of PI3K.

DISCUSSION

In this study, we have demonstrated that beryllium pre-treatment inhibits LPS-mediated IL10 release and induces IL1 β release in primary cells. Furthermore, this effect is greatly enhanced in experiments where we use both the antigen presenting cell and a responder T/B cell mix. Such an effect has been reported recently for IL1 β release by monocytes/macrophages, whereupon direct cellular contact with stimulated T cells plays a role.⁽²⁷⁾ Additionally, we find that beryllium induces intracellular changes in cells, as observed by alterations in phosphotyrosine levels in whole lysates from these cells, inhibition of tyrosine phosphorylation of STAT3, and by the ability of pharmacological inhibitors of PI3K to inhibit IL10 release to much the same extent as beryllium pre-treatment. In all, these results suggest, for the first time, that beryllium alters the ability of human cells to respond to bacterial components by altering intracellular signaling cascades.

While IL10 is normally undetectable in normal healthy human sera or blood cells, changes in IL10 production have been associated with many inflammatory diseases including CBD.⁽³⁰⁾ IL10 production is also thought to alleviate excessive inflammation, and provide protection from endotoxemia.⁽³¹⁾ Our data show that prior exposures to beryllium lead to suppressed IL10 release in response to endotoxin. Furthermore, this suppression is mediated by intracellular signaling events that regulate cell mediated immunity. Huang *et al* have previously reported a function for IL-1 receptor associated kinase (IRAK)1 in regulation of LPS-mediated IL10 expression, *via* phosphorylation of STAT3.⁽³²⁾ IRAK1 deficient splenocytes show no inhibition of IL1 β mRNA upon LPS stimulation. Finally, inhibitors of PI3K have been reported to inhibit STAT3 phosphorylation in primary human cells,⁽³³⁾ suggesting that signaling molecules IRAK1, PI3K and STAT3 may regulate IL10 release upon LPS stimulation in our cells. Consistently, beryllium treated cells show inhibited STAT3 phosphorylation, and pharmacological inhibitors of PI3K mimic beryllium in inhibiting IL10 release.

We show that beryllium induces LPS mediated IL1 β release. IL1 β secretion in response to asbestos and silica is mediated by activation of caspase 1 and the inflammasome complex.⁽³⁴⁾ Nucleotide-binding domain, leucine-rich-repeat containing protein (Nalp) 3 inflammasome is implicated in particulate matter-related pulmonary diseases and hypothesized to play a role as a major proinflammatory “danger” receptor.⁽³⁵⁾ The ability of beryllium to induce IL1 β release suggests the likelihood of such a signaling cascade being activated in these cells, and studies investigating such a mechanism are ongoing.

The effects of various metals on LPS-mediated inflammatory responses have previously been studied both in cells and in animal models. Exposure to lead chloride has been shown to strongly enhance the susceptibility of rodents to endotoxin shock and parasitical infections and in cellular models, lead-treated cells release up to tenfold increased amounts of TNF α , IL-6, IL12, but less IL10 compared to controls.⁽³⁶⁾ Nickel chloride has previously been shown to amplify LPS-triggered IL6 and IL1 β release by activating the Nrf2 antioxidants pathway in human monocytes.⁽³⁷⁾ Mercury potentiates LPS-mediated TNF α expression, decreases IL6 expression and has no effect on LPS-induced IL1 β expression in mouse liver.⁽³⁸⁾ Zinc has a protective effect against LPS/D-galactosamine-induced lethality in mice.⁽³⁹⁾ Thus the ability of various metals to either potentiate or attenuate LPS-mediated inflammatory responses varies, suggesting differential impacts on host immunity.

Bacterial infections have recently been reported to have an impact on triggering metal allergies.⁽⁴⁰⁾ Mice sensitized with nickel and LPS later react to nickel, chromium, palladium and silver.⁽⁴⁰⁾ It is hypothesized that a complex made of LPS and nickel may trigger the immune system, which then sensitizes to any kind of LPS-metal complex. This hypothesis

should allow for the argument that cellular responses to LPS-metal complex are globally different from those to LPS alone. We find that beryllium alters only a subset of the cellular response to LPS. While LPS-mediated IL10 and IL1 β release are altered in the presence of beryllium, IL6 release is unaltered. This suggests that while the immune trigger for these cells is indistinguishable from LPS, beryllium is able to modulate a subset of these responses. Given that certain metals exhibit electrostatic interactions to LPS, ⁽⁴¹⁾ it is however worthwhile to investigate the nature of the LPS-beryllium complexes formed *in vitro*, at physiologically relevant concentrations.

Our results suggest that beryllium may alter the innate immune response of host cells. While a role for TLRs has recently been suggested in metal allergies, ⁽⁴⁰⁾ in CBD, the correlation with incidences of bacterial infections, are for the most part, unexplored. Clinically, pathologically and radiologically, CBD is difficult to distinguish from pulmonary sarcoidosis, ⁽⁴²⁾ and susceptibility to disease for both has been associated with the major histocompatibility complex. ⁽⁴³⁾ Cell wall-deficient bacteria have been found in tissue from patients with sarcoidosis ^(44, 45) and sarcoid BAL fluid reportedly contains notably higher levels of endotoxin. ⁽⁴⁶⁾ Gram negative bacteria are viewed to contribute to the pathogenesis of sarcoidosis, by upregulating expression of IL18. ⁽⁴⁶⁾ In the same study, the authors reported an upregulation of IL18 expression in THP1 cells exposed to BeSO₄ and to a purified protein derivative of *Mycobacterium tuberculosis*, both of which form granulomas as in sarcoidosis. Finally, polymorphisms in innate immune protein, Nod2, have been linked with constitutive NF κ B activation and the development of early-onset sarcoidosis. ⁽⁴⁷⁾ In all, the occurrence of sarcoidosis seems to be somewhat influenced by both genetic susceptibility and environmental factors; and closely linked with bacterial infections. Whether these infections are the cause or effect is still unclear. Along very similar lines, it is likely that bacterial infections will play a role in pathogenesis of “sarcoidosis of known etiology”, ⁽⁴⁸⁾ that is CBD, and our results support that hypothesis. This work warrants future studies involving characterization of endotoxin levels and incidences of bacterial infections in individuals with CBD.

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FIGURE 1.

BeSO₄ alters cytokine release in human PBMC:DC stimulated with LPS. (A) Cells were stimulated with LPS for 24 hours and cell free supernatants were then collected and subjected to IL10, IL1β and IL6 sandwich ELISAs as described in Materials and Methods. Mean ± standard deviation (M ± SD) of LPS stimulated release of IL10, IL6 and IL1β (in pg/mL) is shown. Untreated controls are shown in black and LPS treated cells are shown in grey. Statistical analysis was performed using the Mann-Whitney test (n = 3–5 donors, each sample analyzed in triplicate) (θ denotes p < 0.01 and Ψ denotes p < 0.05). (B–D) Cells were treated with metal sulfate alone for 48 hours (M); metal sulfate for 24 hours followed by LPS for 24 hours (ML), LPS for 24 hours followed by metal sulfate (LM) or co-treated with LPS and metal sulfate (M/L) for 48 hours. Grey bars indicate mock metal treatment (buffer), cross- shaded bars indicate Al₂(SO₄)₃ treated cells and black bars indicate BeSO₄ treated cells. Release of cytokines IL10 (B), IL1β (C) and IL6 (D) are shown as a percentage of control metal treated cells. Statistical analyses were performed using mean values (n = 3–5), each sample analyzed in triplicate. One-way ANOVA was used for comparisons of BeSO₄-treated cells with control-metal treated and Al₂(SO₄)₃-treated cells (# denotes p < 0.001, and * denotes p < 0.01). Mann-Whitney analysis was performed where significance is noted in BeSO₄ treated cells only in comparison to control-metal treated cells (§ denotes p < 0.001).

FIGURE 2.

Cytokine (IL10, TNFα, IL6, IL8, IL12p40 and IL1β (pg/mL)) release from DC in response to LPS and metal sulfates. Cells were treated with metal sulfate alone for 48 hours (M) or metal sulfate for 24 hours followed by LPS for 24 hours (ML). Unshaded bars indicate mock metal treatment (buffer), diagonal-shaded bars indicate Al₂(SO₄)₃ treated cells and horizontal-shaded bars indicate BeSO₄ treated cells. White bars are metal alone (M), and grey bars are metal with LPS treatment (ML). Cells free supernatants were collected and subjected to cytokine sandwich ELISA's as described in Materials and Methods. Statistical analyses were performed using mean values for one donor analyzed in triplicate. One-way ANOVA was used for comparisons of BeSO₄-treated cells with control-metal treated and Al₂(SO₄)₃-treated cells (# denotes $p < 0.001$, and * denotes $p < 0.01$).

FIGURE 3.

Beryllium alters intracellular phosphotyrosine levels and inhibits STAT3 tyrosine phosphorylation in PBMC:DC stimulated with LPS. Cells were treated with metal sulfate for 24 hours followed by LPS for 24 hours (ML). C indicate mock metal control (buffer alone)-treated cells, A indicate $\text{Al}_2(\text{SO}_4)_3$ treated cells and B indicate BeSO_4 treated cells. Cells were separated from supernatant, washed and lysed as detailed in Materials and Methods. Cell lysates were normalized for protein content and subjected to (A) Western blot analyses using anti-phosphotyrosine antibody. Equivalent loading controls are indicated in equivalent bands at 100, 90 and 50 kDa on the blot. Blot is representative of data from three independent experiments. (B) Western blot analyses using anti-phosphotyrosine 705 STAT3 antibody (α pSTAT3). Equivalent total protein is indicated by an anti- β actin blot (lower panel). Blot is representative of data from three independent experiments. (C) Quantitation of pSTAT3 levels in whole lysates. Densitometric measurements were made on blots that were imaged using the ChemidocXRS system and Quantity One® software. The percentage inhibition was calculated relative to control LPS-treated samples (CL).

FIGURE 4.

Inhibitors of PI3K inhibit IL10, but have no effect on IL6 or on IL1β secretion. Cells were treated with LPS where indicated; in the presence of BeSO₄, Al₂(SO₄)₃, 5 nM wortmannin or 5 μM LY294002 as indicated. Cells free supernatants were collected and subjected to cytokine sandwich ELISA's as described in Materials and Methods. Mean (± SD) pg/mL of detected IL10 are represented using black bars, IL6 with clear bars and IL1β with grey bars. Each sample was assayed in triplicate and results are representative of three independent experiments.