Elevated Prostaglandin E_2 Post-Bone Marrow Transplant Mediates Interleukin-1β Related-Lung Injury

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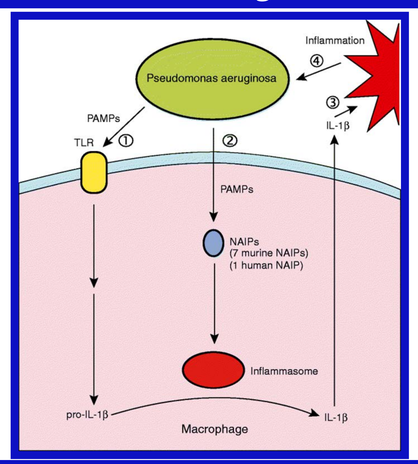
Abstract

Every year in the United States, more than 18,000 patients undergo hematopoietic stem cell transplantation (HSCT) as a way to treat malignant disease, such as cancer, or nonmalignant inherited disorders. Unfortunately, it is expected that 60% of HSCT patients will develop lung complications due to infections caused by a wide array of pathogens. One of the most successful lung bacterial pathogens that invade HSCT recipients is *Pseudomonas aeruginosa*. Successful colonization of this pathogen within the lung compartment can have many negative effects in the host including mortality and development of lung injury. Researchers have shown that Interleukin-1β (IL-1β) release by alveolar macrophages (AMΦs) after *P. aeruginosa* infection facilitates bacterial colonization as well as provokes IL-1β-mediated lung injury. In this study, we show that overexpression of prostaglandin E_2 (PGE_2) post-HSCT, signals via EP2 or EP4 to induce cyclic adenosine monophosphate (cAMP). This activates protein kinase A or the exchange protein activated by cAMP (EPAC) to induce transcription of IL-1β. The processing of IL-1β post- *P. aeruginosa* occurs via either canonical (caspase-1 mediated) or non-canonical (caspase-8 mediated) inflammasomes. Furthermore, PGE_2 can limit autophagy in alveolar macrophages to impair bacterial killing. Elevations of PGE_2 correlate with increased IL-1β and evidence of acute lung injury. Thus, elevated PGE_2 post-HSCT promotes lung injury and impaired bacterial killing; moreover, cyclooxygenase inhibitors show therapeutic benefit in HSCT recipients.

Background

- More than 18,000 hematopoietic stem cell transplants (HSCT) are performed yearly in the United States
- HSCT patients become susceptible to pulmonary infections against *Pseudomonas aeruginosa*
- In February 2017, the World Health Organization reported that *P.aeruginosa* is a critical pathogen for which new therapeutic strategies are needed
- *P. aeruginosa* infection causes life threatening effects in HSCT patients & immunocompromised individuals
- Exacerbated levels of IL-1β secreted by AMΦs post-*P. aeruginosa* infection leads to lung tissue injury
- Mice that have been depleted of AMΦs prior to *P. aeruginosa* infection experience less acute lung injury and improved bacterial killing compared to wild type mice
- AMΦs in HSCT patients express high levels of Cyclooxygenase-1/2 enzymes and secrete elevated levels of PGE_2
- PGE_2 has both anti and pro inflammatory effects
- The role of PGE_2 in regulation of IL-1β is unknown and contradictory

Pseudomonas aeruginosa and IL-1β



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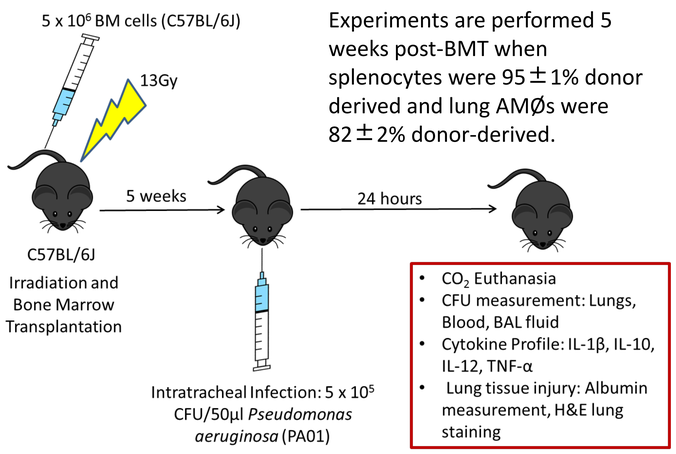
P. aeruginosa Induced Acute Lung Injury

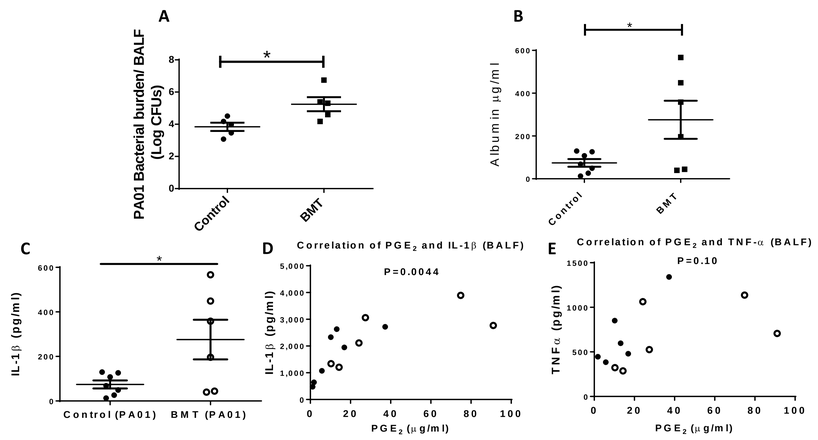


Experiments are performed 5 weeks post-BMT when splenocytes were 95±1% donor derived and lung AMØs were 82±2% donor-derived.

- CO_2 Euthanasia
- CFU measurement: Lungs, Blood, BAL fluid
- Cytokine Profile: IL-1β, IL-10, IL-12, TNF-α
- Lung tissue injury: Albumin measurement, H&E lung staining

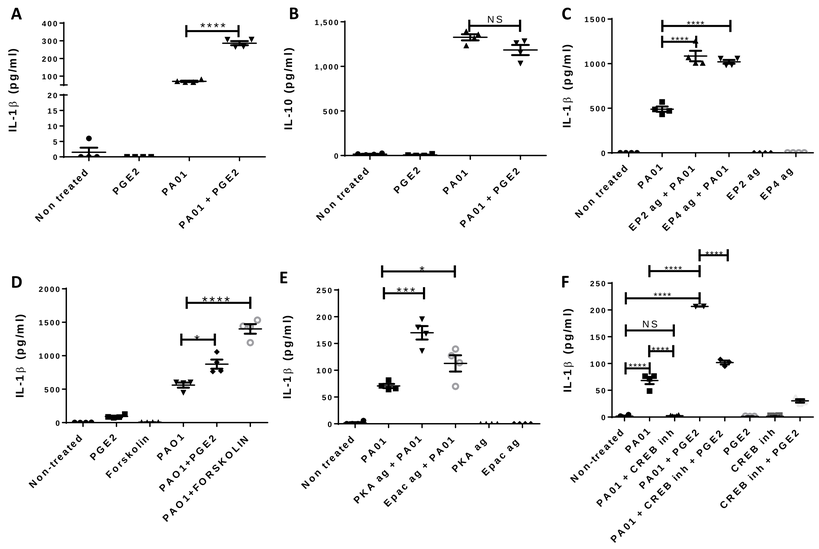
Results

BMT Mice are Deficient in Clearing *Pseudomonas aeruginosa* Infection and Experience Exacerbated Lung Tissue Injury



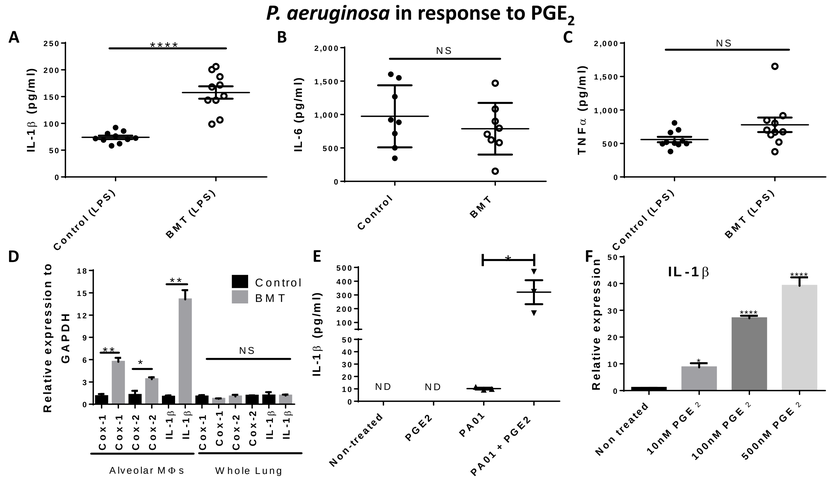
(A) Colony Forming Units (CFUs) of infected non-transplanted control and BMT mice with $5x10^5$ CFUs of *P. aeruginosa* (PA01). (B) Albumin measurements from bronchoalveolar lavage fluid (BALF) from PA01 infected control and BMT mice. (C) Measurements of IL-1β in control and BMT mice. (D and E) Correlations between IL-1β and PGE_2 or PGE_2 and TNF-α of infected mice. In panels D and E, closed circles are control mice and open circles are BMT mice.

PGE_2 mediated increase in IL-1β is dependent on activation of transcription factor CREB by increasing levels of cAMP dependent on EP2 and EP4 signaling



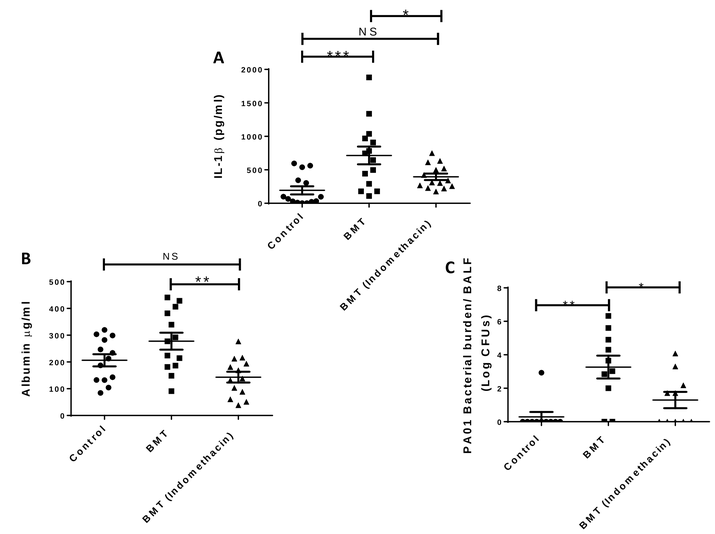
(A-F) IL-1β or IL-10 measurements from supernatant of bone marrow-derived MØs (BMDMs) treated or not with PGE_2(100nM), Forskolin (25µM), CREB inhibitor (100µM, Naphthol AS-E phosphate), and/or agonists for EP2 (1µM, Butaprost), EP4 (500nM, ONO-AE1-329) , PKA (50µM, 6-BNZ-cAMP), or Epac (50µM, 8-pcpt-2′-OM-cAMP) while infected or not with *P. aeruginosa*, MOI:10.

Alveolar Macrophages in BMT mice Account for higher IL-1β Release post-*P. aeruginosa* in response to PGE_2



(A) IL-1β , (B) IL-6 and (C) TNF-α measurements by ELISA from BALF of LPS (50ug) treated control and BMT mice.(D) RTqPCR measurement of relative gene expression of Cox-1, Cox-2, and IL-1β from AMs and whole lung cells from uninfected control and BMT mice normalized to GAPDH (n=3 control; n=3 BMT/group).(E) IL-1β measurements by ELISA from AMØs infected or not in vitro with PA01 (MOI:10), treated or not with 100nM of PGE2. (F) Relative IL-1β gene expression after PGE_2 stimulation (n=3/group).

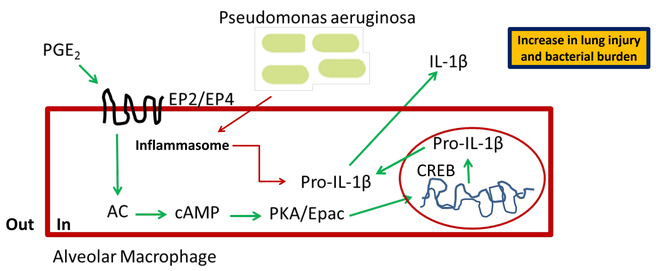
Decreasing Levels of PGE_2 by Indomethacin Treatment Leads to Decreased IL-1β in the Lung post *P. aeruginosa* Infection in BMT mice



A) IL-1β and (B) albumin measurements from the BALF of control, and BMT mice treated or not with Indomethacin for 24 hours after PA01 infection; measurements done by ELISA; (C) PA01 colony forming unit measurement in BAL from infected and indomethacin-treated mice, 24 hours post-infection.

Conclusions

- Bone marrow transplant mice experience exacerbated IL-1β-mediated lung injury post-*P. aeruginosa* infection
- Elevated levels of PGE_2 increase IL-1β in BMT mice
- PGE_2 increases IL-1β via EP2 and EP4 stimulation
- AMØs from BMT mice secrete higher levels of PGE_2 and have elevated levels of IL-1β transcripts compared to control mice
- PGE_2 inhibits autophagy-mediated *P. aeruginosa* killing (data not shown) and exacerbates lung injury via induction of IL-1β post-BMT
- Inhibition of COX-1/2, prior to *P. aeruginosa* infection decreases lung injury, IL-1β and improves bacterial clearance



Future Directions

- Perform chromatin immunoprecipitation assays in PGE_2 stimulated AMØs to determine the master transcription factor necessary for the increase of IL-1β but not IL-10, IL-6, or TNF-α
- Improve bacterial killing and lung homeostasis post-*Pseudomonas aeruginosa* infection by using selective inhibitors of the PGE_2 signaling pathway.
- Survival assays (with Anakinra treatment in wild type and BMT mice, and compare to indomethacin treatment in wild type and BMT

References

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