Interactions between the IFN-g/IL-27 and COX-2/PGE2 pathways within the tumor microenvironment: implications for cancer immunotherapy with anti-PD-(L)1

Seoho Lee, Shuming Chen, Tracee L. McMiller, Isaac Morales, and Suzanne L. Topalian

Medical Student Research Symposium

Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

BACKGROUND

- Efficacy of targeted immune checkpoint inhibitors (ICIs) such as anti-PD-(L)1 in cancer treatment has been widely variable.[1]
- Cyclooxygenase 2 (COX-2) protein is highly expressed in some cancer types with low response rates to ICI.[2]
- COX-2 induces PGE2 secretion, which can hinder anti-tumor immunity. Inhibitors can target various steps within this pathway.
- COX-2/PGE2 and IFN-g/IL-27 activity within the tumor microenvironment (TME) can alter response to ICI.[3]
- We have previously shown that the COX-2/PGE2 and IFN-g/IL-27 pathways are critical in modulating myeloid cell functions.[4]

STUDY OBJECTIVES

- The purpose of this study was to characterize the interactions between the pro-inflammatory IFN-g/IL-27 and immune-inhibitory COX-2/PGE2 pathways within human myeloid cell populations.

MATERIALS AND METHODS

- **COX-2 expression and PGE2 secretion:** Normal donor (ND) human peripheral blood monocytes (Monos), immature monocyte-derived dendritic cells (imDCs), and the human monocytic leukemia cell line THP-1 were treated with toll-like receptor (TLR) agonists (TLR2, 4) or TME-resident cytokines associated with high PD-L1 and COX-2 expression (IL-1a, IL-1b), and simultaneously treated with exogenous IFN-g or IL-27. COX-2 protein was detected by intracellular flow cytometry. PGE2 secretion was quantified using ELISA.
- **PGE2 and IFN-g/IL-27 signaling:** Monos were treated with IFN-g with or without PGE2. Monos treated with IFN-g and PGE2 were treated with inhibitors of EP2, EP4, or both (EP2/4i). STAT1/pSTAT1 protein were detected using Western blotting and PD-L1 expression was measured using flow cytometry.

RESULTS

FIGURE 1. In Monos, IFN-g/IL-27 counteracted some, but not all, cytokine- or TLR-induced COX-2 expression while upregulating PD-L1. PGE2 secretion correlated with COX-2 expression. Cytokines and TLR-agonists varied in degree of COX-2 and PD-L1 induction across 2-3 NDs. * p ≤ 0.1; ** p ≤ 0.01; *** p ≤ 0.001.

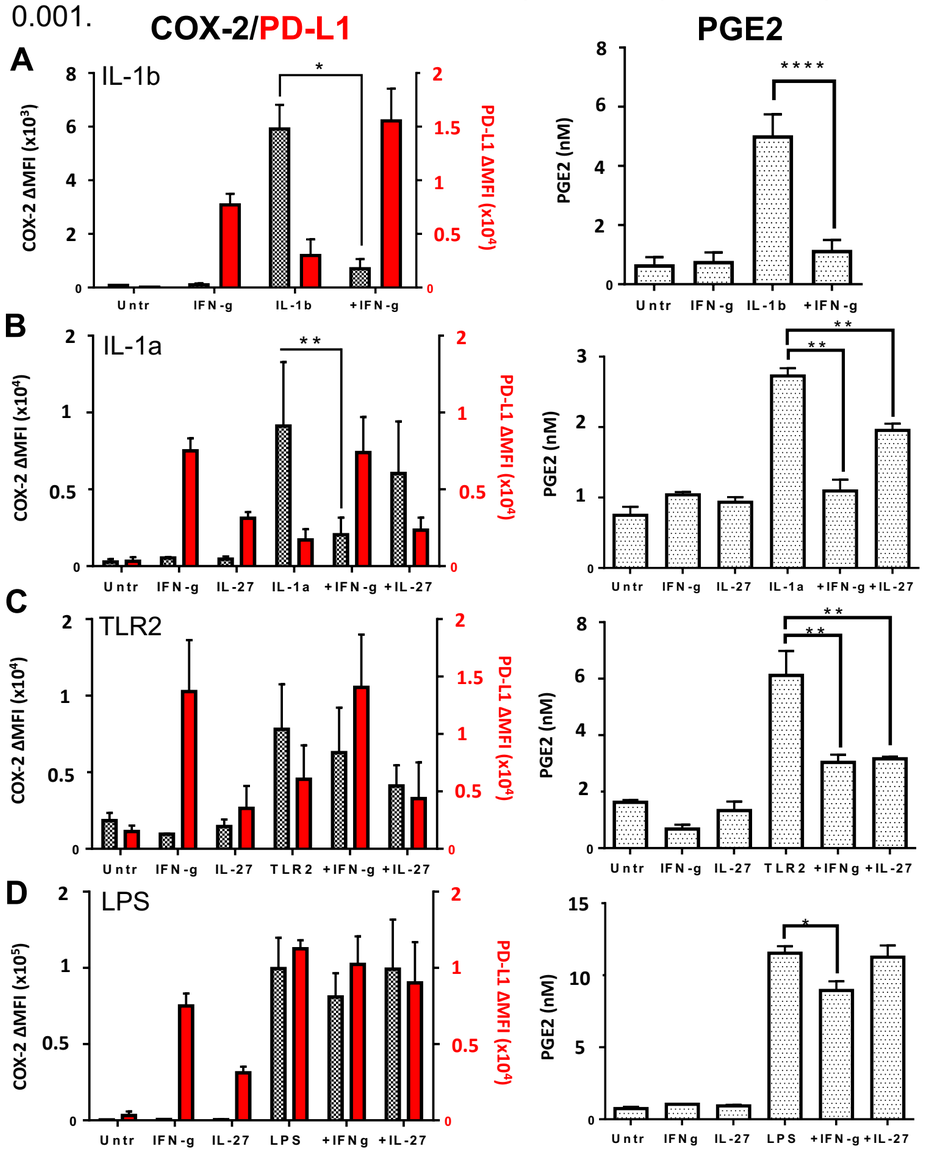


FIGURE 2. In imDCs, IFN-g/IL-27 had minimal effects on most cytokine- or TLR-induced COX-2 but upregulated LPS-induced COX-2. Data representative of 3 NDs. ΔMFI (left) and FACS histograms (right).

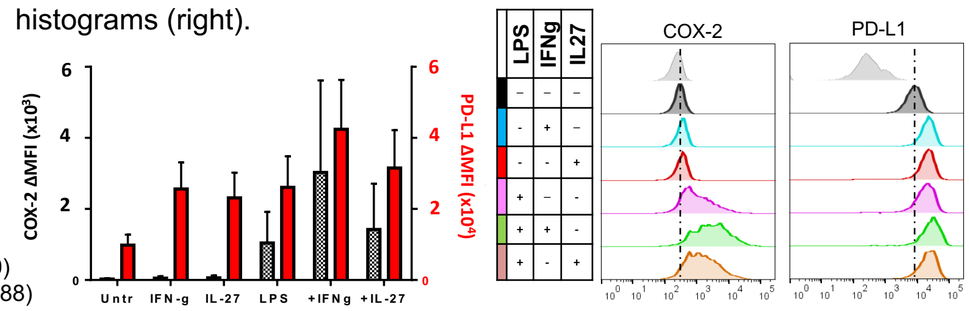


FIGURE 3. In THP-1 cells, IFN-g/IL-27 upregulated most cytokine- or TLR-induced COX-2 and PD-L1. LPS treatment ΔMFI (left) and FACS histograms (right).

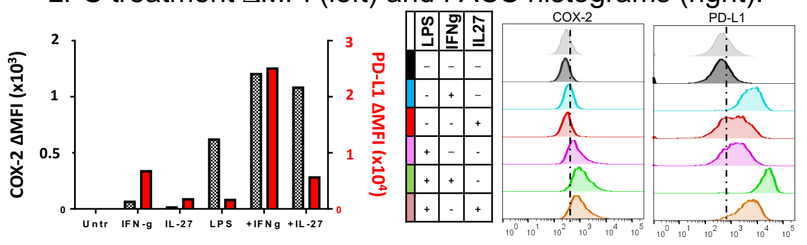
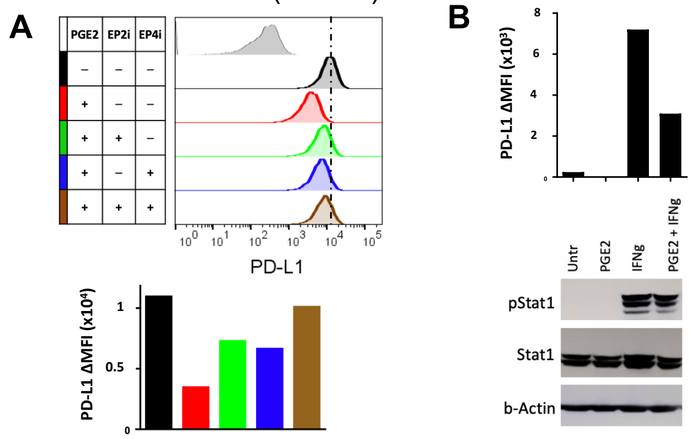
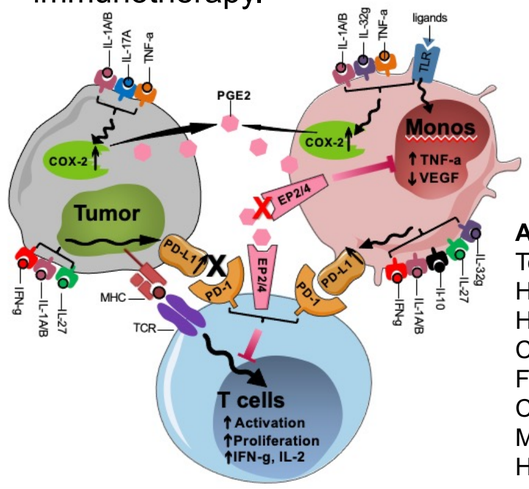


FIGURE 4. In Monos, PGE2 suppressed IFN-g induced PD-L1 expression, which can be restored by EPi, but utilized mechanisms separate from pSTAT1. A) EP2/4i treatment **B)** ΔMFI PD-L1 (top) and STAT1 western blot (bottom).



CONCLUSIONS AND IMPLICATIONS

- COX-2 and PD-L1, both capable of mediating immunosuppression in the tumor microenvironment, are non-redundant in certain myeloid cell populations. This supports the use of COX-2/PGE2 inhibitors in combination with anti-PD-(L)-1 to enhance the efficacy of cancer immunotherapy.



Acknowledgements: Topalian Lab, Dr. Marc Halushka, Johns Hopkins Scholarly Concentration, Dean's Funding, NCI R01-CA142779, Bristol Myers Squibb, Johns Hopkins BKI

References:

1. Kalbasi et al., *Nat. Rev. Immunol.* (2020)
2. Besharati et al., SITC 2018 (abstr)
3. Bonavita et al., *Immunity* (2020)
4. Chen et al., JITC 2021 (abstr 288)