POSTER TITLE

Immune checkpoint inhibitors promote colitis via the upregulation of adhesion molecules in an experimental murine model

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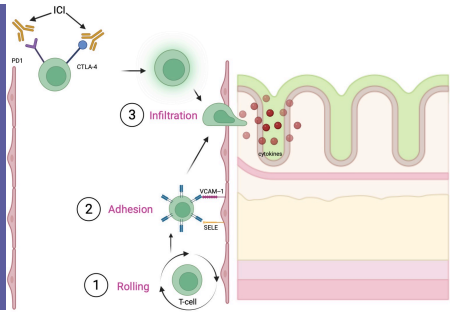
ABSTRACT

Immunotherapy for cancer using immune checkpoint inhibitors (ICI) is a common and effective way to treat a plethora of cancers. However, the use of ICIs may lead to multiple types of immune-related adverse events (irAEs) including colitis. Studying various biomarkers including adhesion molecules may allow better management of adverse immune events in patients receiving immunotherapy. The effects of ICI on adhesion molecules were studied using a mouse model. One group of mice was injected with anti- CTLA4 and anti-PDL1 (ICIs) and another was injected with the isotype control (ISO). Following the administration of ICI or Isotype, 2% Dextran Sodium Sulfate (DSS) was administered in the drinking water. Immunohistochemistry (IHC) was then performed on the colon sections to evaluate the expression of adhesion molecules: VCAM-1 and E-Selectin. It was shown that colons from mice who received an ICI + DSS treatment had more severe forms of colitis evident by the presence of more positive signals of VCAM-1 and E-selectin on vascular endothelial cells and increased immune cell infiltration into the lamina propria of the distal colon. Immunocytochemistry (ICC) was also conducted on Phosphorylated-Focal Adhesion Kinase (P-FAK) and Phosphorylated-Paxillin (P-Pax) using Human Umbilical Vascular Endothelial Cells (HUVEC) to study the signaling pathways between adhesion proteins under different microenvironment pH conditions. The cell culture studies showed that at a pH of 6.4 the P-FAK and P-Pax tend to accumulate on the periphery of the cell and actin stress fibers are located in the center of the cell. This is different from physiological pH where the adhesion regulatory proteins accumulate within the cell body and the stress on actin fibers is not as severe when compared to a more acidic environment. These findings may prove that cell adhesion is impacted by changes in the microenvironment. Comparing these findings shows that immune checkpoint inhibitors exacerbate colitis by causing upregulation of adhesion molecules.

Treating cancer patients with immune checkpoint inhibitors may cause colitis and other advesre immune events by upregulating adhesion molecules.



BACKGROUND

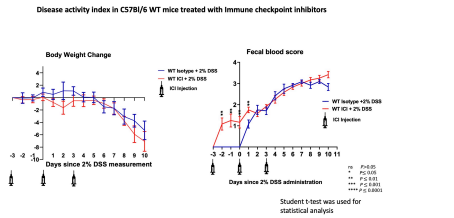


Fig 1. Prior results obtained by the Yang Lab of comparisons in changes in body weight and amount of blood in feces when injected with WT ICI + 2% DSS, WT ISO + DSS or ICI.

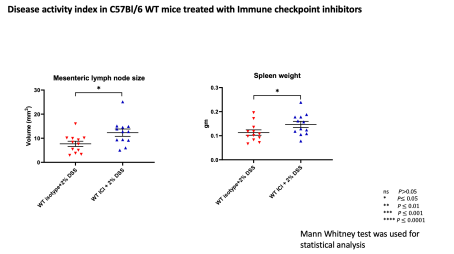
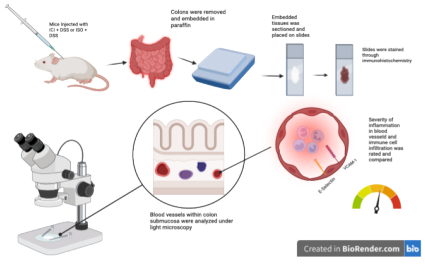


Fig 2. Prior results obtained by the Yang lab determining disease severity by comparing mesenteric lymph node size and spleen size when injected with WT ICI + 2% DSS or WT ISO + 2% DSS.

METHODS

- Mice were treated with ICI + DSS or ISO + DSS to induce colitis.
- Mouse colons were obtained and embedded for sectioning.
- Sectioned mouse colons were placed on slides.
- Slides were stained using IHC including an antibody to the relevant adhesion molecule (VCAM-1, E-Selectin)
- Stained slides were rated on severity (mild, moderate, or severe) based on positive signals on blood vessels or infiltration of immune cells.
- ICC was performed on FAK and Paxillin to observe phsophorylation patterns.



RESULTS

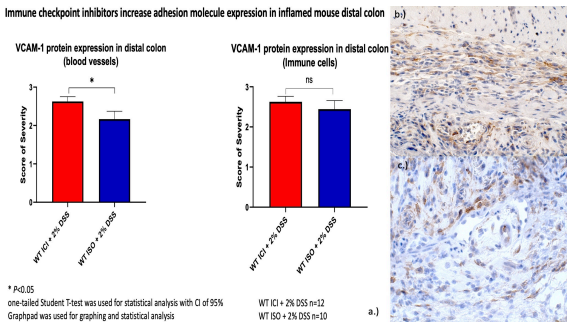


Fig 3. Results of VCAM antibody. a. Severity of inflammation based on positive signals of VCAM-1 expression on blood vessels and immune cells (1-mild, 2-moderate, 3-severe). **b.** Representative picture of distal colon of mouse treated with ISO + 2% DSS. **c.** Representative picture of distal mouse colon treated with ICI + 2% DSS.

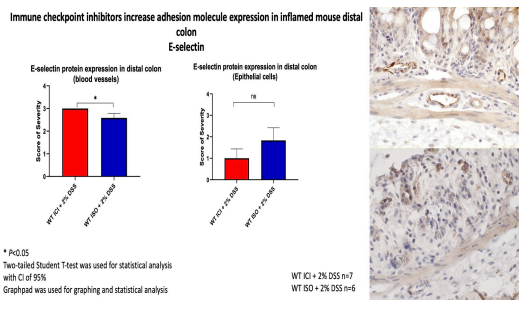


Fig 4. Results for E-Selectin Antibody. **a.** Severity of inflammation based on positive signals of E-Selectin on blood vessels and epithelial tissue (1-mild, 2-moderate, 3-severe). **b.** Representative image of distal colon of mouse treated with ISO + 2% DSS with E-Selectin antibody. **c.** Representative image of mice treated with ICI + 2% DSS with E-Selectin antibody.

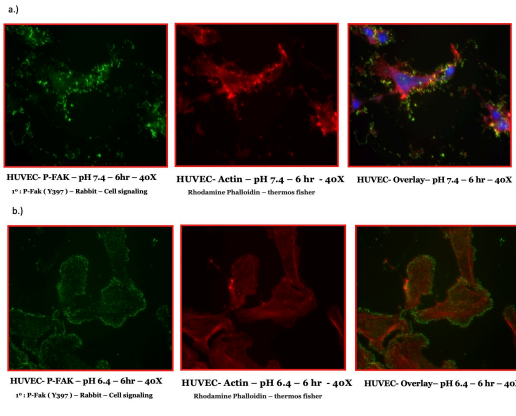


Fig 5. Expression of phosphorylated-focal adhesion kinase in different microenvironments. a. Representative image of P-FAK expression in pH of 7.4 with actin expression and overlay.**b.** Representative image of P-FAK expression at pH of 6.4 with actin and overlay.

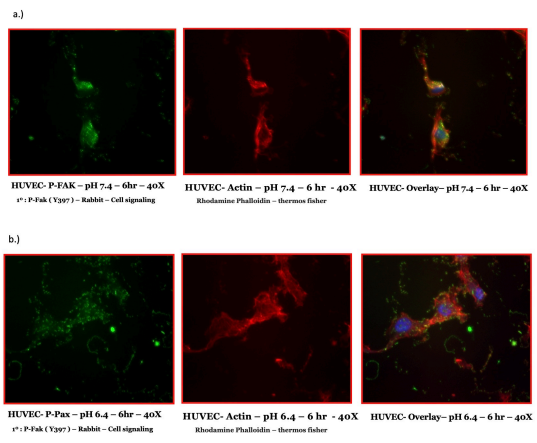


Fig 6. Expression of phosphorylated-paxillin in different microenvironements. a. Representative image of P-Pax in pH of 7.4 with actin expression and overlay.**b.** Representative image of P-Pax in pH of 6.4 with actin and overlay.

REFERENCES

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2. Lim, Ssang-Taek, et al. "Nuclear-localized focal adhesion kinase regulates inflammatory VCAM-1 expression." Journal of Cell Biology 197.7 (2012): 907-919.

ACKNOWLEDGEMENTS

- Summer Scholars Research Program
- Research Grants from the National Institute of Health

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