Title: Lymphocyte activation gene (LAG)-3 on T cells is a potential therapeutic target in ulcerative colitis

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Background: LAG-3 is a transmembrane protein expressed on T cells following antigen-driven activation. Although it functions as a negative co-stimulatory receptor, similar to PD-1 and CTLA4, its expression identifies lymphocytes that may contribute to initiation and persistence of inflammation in patients with inflammatory disease. We quantified LAG-3 expression in patients with ulcerative colitis before and after treatment, and characterised the sub-populations of activated T cells in the mucosa that express LAG-3, examining in particular effector cells, memory cells, and regulatory cells. Previously we presented an initial characterization of LAG-3^+ T cells[1], here we extend our findings with qPCR and functionally characterize lamina propria LAG-3^+ T cells with intracellular cytokine staining.

Methods: High-dimensional flow cytometric analysis on blood and inflamed and non-inflamed colonic biopsy samples from patients with ulcerative colitis (n=42) and non-IBD controls (n=9) was performed. Immunohistochemical analysis on mucosal samples was used to determine the correlation of LAG-3^+ cells with endoscopic and histological scores, as well as the impact of biologic therapies. Cytokine production from mucosal LAG-3^+ cells was determined by flow cytometry and quantitative RT-PCR.

Results: The frequency of LAG-3^+ cells in peripheral blood was negligible (<0.5%), regardless of disease activity in patients. However, in the lamina propria, the frequency of LAG-3^+ T lymphocytes was markedly increased in active UC compared with uninflamed and non-IBD controls ($p<0.0001$ and $p=0.001$, respectively) and correlated positively with endoscopic score (UCEIS,$p=0.004$, $r=0.43$). LAG-3 expression was enriched on effector memory and CD161$^+$ T cells. *LAG3* mRNA levels were also increased in active disease ($p=0.003$ and $p=0.008$, respectively) and correlated with the histological Nancy score ($p<0.001$, $r=0.68$). Mucosal LAG-3^+ T cells demonstrated robust production of IFNγ ($p=0.04$) and IL-17A ($p=0.01$) when stimulated *ex vivo* compared to LAG-3^- cells, with lower amounts of IL-10 detected ($p<0.06$). In patients undergoing treatment for ulcerative colitis, the number of LAG-3^+ cells decreased in patients who responded to therapy ($p<0.0001$, n=11), but remained elevated in non-responders ($p=0.058$, n=12).

Conclusion: LAG-3 expression is not altered in circulating blood. However, mucosal expression is increased in inflammation and normalises after successful treatment. Although some reports suggest LAG-3^+ cells have mainly regulatory functions, in human IBD, LAG-3^+ cells are mainly effector memory cells and predominantly produce IFNγ, IL-17A and low levels of IL-10. Therefore, depleting LAG-3^+ cells is a promising strategy for IBD that merits further clinical investigation.

1. Slevin S., Tan M., Lahiff C., Williamson K., Geremia A., Hughes S., Leavens K., Nevin K., Marks D.J.B., Tarzi R., Srinivasan N., Arancibia C., Keshav S. Intestinal expression of LAG-3 correlates with inflammatory activity and response to biological therapy in ulcerative colitis. ECCO 2018. Available at: https://www.ecco-ibd.eu/publications/congress-abstract-s/abstracts-2018/item/p064-intestinal-expression-of-lag-3-correlates-with-inflammatory-activity-and-response-to-biological-therapy-in-ulcerative-colitis.html.