Anti-α4β7 antibody reduces intestinal myeloid cell turnover in SIV-infected rhesus macaques

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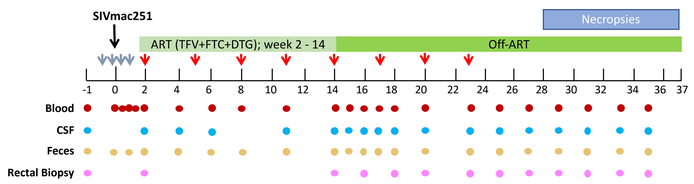
BACKGROUND

While cART successfully suppresses viral replication in HIV-infected individuals, treatment interruption (ATI) results in rapid rebound of active virus. Further, even during cART suppression, many individuals experience chronic immune activation due to ongoing microbial translocation across the gut barrier. This chronic inflammation shares many hallmarks of inflammatory bowel diseases (IBD), and studies suggest that administration of a primatized anti-$\alpha_4\beta_7$ integrin homolog to the humanized monoclonal antibody vedolizumab (Entyvio) approved to treat IBD may offer a therapeutic benefit to SIV-infected rhesus macaques[2].

Early understandings of vedolizumab suggested its mechanism of action depended on blocking inflammatory lymphocytes from trafficking to the gut endothelium expressing the addressin MADCAM-1 thereby preventing further damage. However, emerging evidence has since implicated myeloid cells, not lymphocytes, in modulating disease progression[3]. Further, studies to predict efficacy of vedolizumab have found that patients with a higher relative abundance of BPB like *Roseburia* and *Faecalibacterium* are more likely to achieve remission[4]. Here, we hypothesized anti-$\alpha_4\beta_7$ antibody may have similar function in SIV-infected macaques and whether these mechanisms from IBD patients treated with vedolizumab can provide insight into the role of gut immune trafficking in the pathogenesis of SIV/HIV infection.

METHODS

Prior to infection, nine rhesus macaques (*Macaca mulatta*) were depleted of CD8 cells by intravenous administration of anti-CD8 antibodies on Days -4, -1, 0, 3, and 6. All monkeys were infected on Day 0 by intravenous (IV) administration of SIVmac251. On Day 14 of acute infection, cART (TFV + FTC + DTG) administration was initiated and continued for 12 weeks. Also on Day 14, the monkeys were separated into two groups: one treated with primatized anti-$\alpha_4\beta_7$ monoclonal antibodies and the other with rhesus IgG. Antibodies were administered every three weeks for a total of eight infusions. At the end of study, all macaques were humanely euthanized and tissues and feces collected for further analysis. Immune cells isolated from the duodenum and ascending colon by enzymatic digestion, filtration, and density gradient centrifugation. Flow cytometry was performed and the Bujko gating strategy was used to determine macrophage maturity. RNA was isolated from the duodenum and ascending colon, and tissue viral loads were determined by qPCR. DNA was isolated from the feces, 16S rRNA sequencing performed, and the relative abundance of butyrate-producing was determined by the ratio of butyrl-CoA transferase to 16S rRNA gene copies using degenerate primers and qPCR.



1) Anti-$\alpha_4\beta_7$ mAb significantly ***decreases*** monocyte infiltration, immature macrophage, and CD103+ dendritic cells in the small intestine lamina propria.
2) This ***reduction*** was associated fecal butyrate-producing bacteria and ***tissue viral loads***.
3) Butyrate-producing bacteria may be a key factor in anti-$\alpha_4\beta_7$ efficacy during SIV infection.

RESULTS

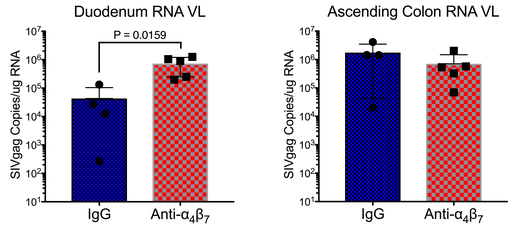


Fig. 1. Viral Loads: Anti-$\alpha_4\beta_7$ was associated with greater viral loads in the small intestine, the site of MADCAM-1 expression, suggesting a complex immune dynamic associated with therapy.

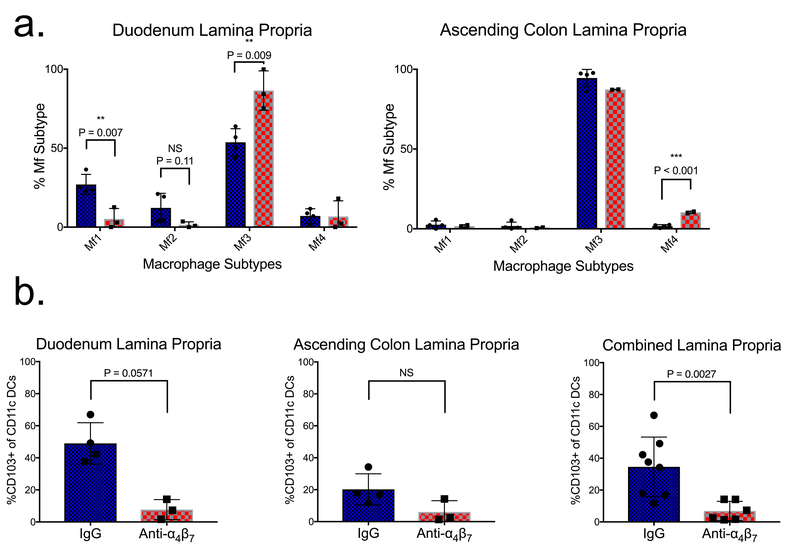


Fig. 2. Tissue Myeloid Turnover: a) Bujko's gating strategy determines gut macrophage maturity with Mf1 representing recently-differentiated monocytes; Mf2, intermediates; and Mf3, mature mucosal macrophages. Mf4 are muscularis macrophages. Anti-$\alpha_4\beta_7$ was associated with decreased macrophage turnover in the duodenum. b) Additionally, anti-$\alpha_4\beta_7$ was associated with lower retinoic acid-producing CD103+ dendritic cells.

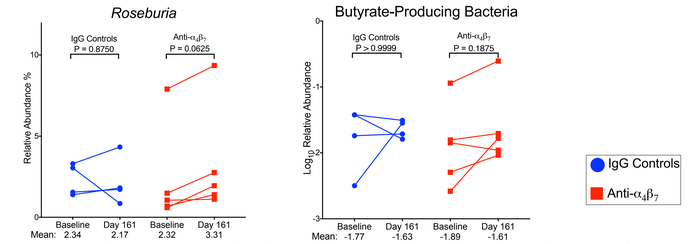


Fig. 3. Butyrate-Producing Bacteria (BPB): Four anti-$\alpha_4\beta_7$-treated animals started with a lower baseline relative abundance of *Roseburia* compared with controls. Following therapy, all five experimental animals had an increase in the relative abundance of *Roseburia*.

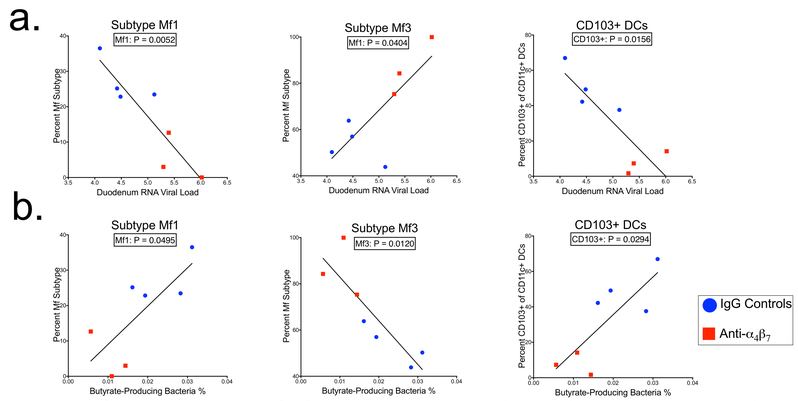


Fig. 4. Myeloid Turnover Associations: a) Duodenum tissue viral loads are associated with lower macrophage turnover and CD103+ DCs. B) BPB were associated with increased macrophage turnover and increased CD103+ DCs.

CONCLUSIONS AND FUTURE DIRECTIONS

- BPB may be an independent variable in efficacy of anti-$\alpha_4\beta_7$ therapy during SIV infection, as has been demonstrated with vedolizumab efficacy in humans with IBD, and this may be mediated by myeloid cell turnover.
- Ongoing analysis on the CNS myeloid and other immune cells will help elucidate the role of gut macrophage turn over and its connection with BPB in modulating the gut-brain axis during SIV infection with and without anti-$\alpha_4\beta_7$ therapy.
- Future studies should focus on understanding the role of the microbiome on pathogenesis of HIV/SIV infections which primarily target immune cells in the gut. Further, our data presented here suggests a role in microglia-like muscularis macrophages in pathogenesis.

REFERENCES

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