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IN PERSON & ONLINE

# REAL-TIME INHIBITION OF INTEGRIN α4β7 BY A SMALL MOLECULE INHIBITOR IMPAIRS B LYMPHOCYTE ADHESION IN MURINE PEYER'S PATCHES

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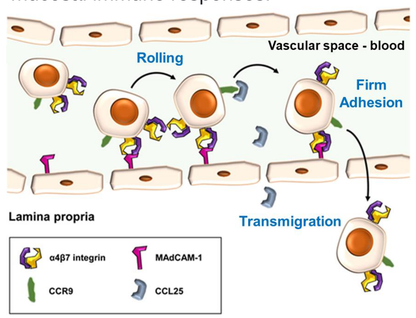
## INTRODUCTION

The inhibition of α4β7 integrin is a clinically proven mechanism for the treatment of inflammatory bowel disease (IBD). Leukocyte trafficking to gut-associated lymphoid tissues (GALT) such as the Peyer's patches (PP) is mediated by the interaction of α4β7 with its ligand mucosal addressin cell adhesion molecule 1 (MAdCAM-1, Figure 1). The PP serve as immune inductive sites responsible for the generation of mucosal immune responses.




Vedolizumab, an anti-α4β7 antibody approved for the treatment of IBD, blocks leukocyte trafficking to the gut. Previously, murine studies showed that α4β7 blockade results in PP decellularization[1,2]. The current study investigates the real-time mechanistic effect of MT-108, a potent and selective small molecule α4β7 inhibitor, on B cell trafficking to PP mucosal endothelial cells.

**Figure 1.** Lymphocyte trafficking in the gut is mediated by α4β7. (Adapted from Zundler et al., 2017)

## METHODS

**Spinning Disk Intravital Microscopy (IVM):** Naïve C57/BL6 mice were anesthetized by intraperitoneal injection of 200 mg/kg of ketamine and 10 mg/kg of xylazine. A catheter was placed in the jugular vein to allow for intravenous (i.v.) access. The fluorophore-conjugated antibodies CD19-AF647, for labeling all B cells, and CD31-AF488, to label the vasculature, were injected intravenously. The small intestine was externalized, and the PP were imaged via an optimized GI imaging chamber (Figure 2) using a spinning disk confocal microscope with an Olympus BX51W1 base fitted with a 10x/0.5 SFluor air objective. Laser excitation at 491 nm and 640 nm was used and fluorescence in the GFP and Cy5 channels was visualized with the appropriate long pass filter (536±40 nm and 692±40 nm respectively). Exposure and sensitivity settings were maintained at the same level for all experiments.

After initial recording of the PP vessels at baseline (starting at 0 minute), the mice were intravenously administered small molecule α4β7 inhibitor MT-108 (1, 3, or 10 mg/kg) or anti-α4β7 blocking antibody DATK32 (300 µg per mouse). The PP vessels, for time intervals of 5-7 minutes, were recorded at 30 and 60 minutes after treatment was administered.

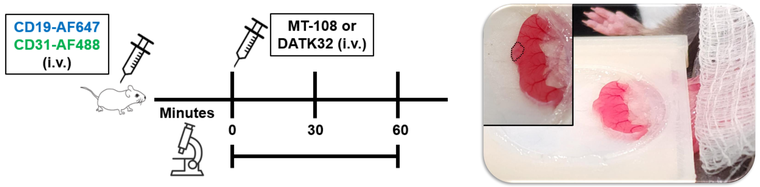



**Figure 2.** Schematic for the intravital microscopy of mouse Peyer's patches.

**Intravital Imaging Analysis:** For analysis, several venules/PP over a 100 µm length section per vessel were identified and quantified in each mouse. The number of rolling, adherent, as well as the velocity, of B cells at the indicated time points was determined manually offline during video playback analysis using Volocity software (version 6.0.1).

## RESULTS

### Visualization of α4β7 small molecule inhibitor blocking B cell trafficking within Peyer's patches.

(A) MT-108 (B) Vehicle

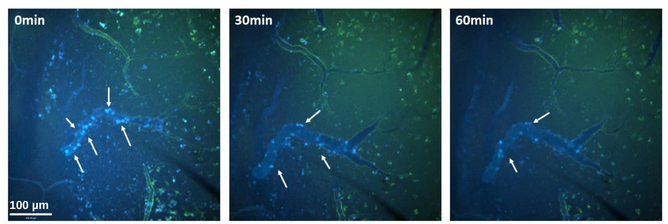
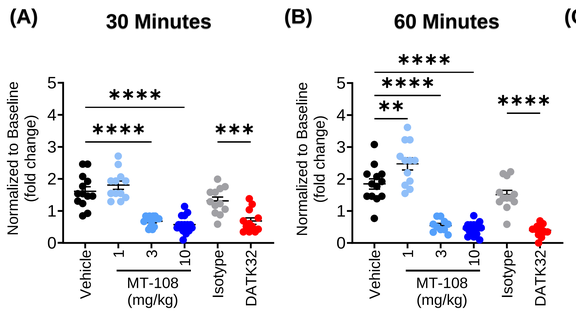



**Figure 3.** Representative PP intravital microscopy videos of MT-108 (10 mg/kg) (A) or vehicle (B) treated mice. The videos were compressed to 5 fps (frames per second). (C) Representative PP intravital microscopy images of mice before (0 min) or 30 and 60 minutes after MT-108 treatment. Images depict B cells (blue, indicated by arrows) binding to PP endothelial cells (green) within a 60 minute time frame.

### B cell adhesion to endothelial cells is significantly reduced as early as 30 minutes post α4β7 inhibition.




| MT-108 Dose (mg/kg) | IC$_x$ (Mean ± SD) |
|---|---|
| 1 | 80 ± 25 |
| 3 | 88 ± 10 |
| 10 | 94 ± 7 |

**Figure 4.** B cell adhesion to endothelial cells 30 (A) or 60 (B) minutes after treatment. Results for each group were normalized to the average of mean adhesion at baseline (0 min). Data are mean ± SEM, analyzed by one-way ANOVA with Holm-Sidak multiple comparisons test, ****p <0.0001, ***p <0.001 **p <0.01 relative to their respective vehicle or isotype control (n = 12-17 vessel length sections, 4-5 mice per group). (C) IVM MT-108 plasma exposures at 60 minutes post-administration expressed as steady-state PP decellularity inhibition constants derived from historical data[1].

### Lymphocyte Trafficking Metrics

Adherence: The number of B cells which adhere to the 100 µm section for ≥30 seconds during the recording time (cells/100 µm).

Rolling Velocity: The time it takes a B cell to travel through a 100 µm vessel length section (expressed as speed in µm/s).

Rolling Flux: The number of B cells passing through a 100 µm vessel section in one minute (cells/min).

### α4β7 blockade increases the rolling velocity and flux of B cells in endothelial vessels in a dose-dependent manner.

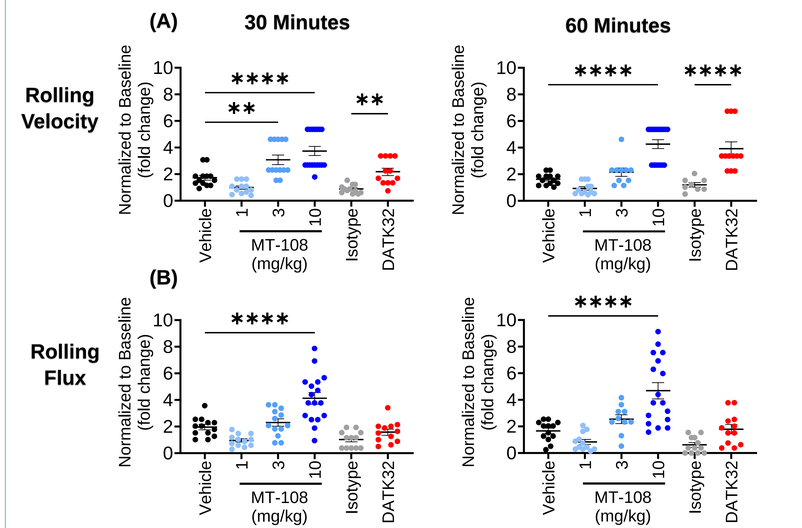



**Figure 5.** B cell rolling velocity (A) and rolling flux (B) in endothelial vessels 30 (left) or 60 (right) minutes after treatment. Results for each group were normalized to the respective averages of mean rolling velocity or rolling flux at baseline (0 min). Data are mean ± SEM, analyzed by one-way ANOVA with Holm-Sidak multiple comparisons test, ****p <0.0001, **p <0.01 relative to their respective vehicle or isotype control (n = 12-17 vessel length sections, 4-5 mice per group).

## CONCLUSIONS

- Intravital microscopy techniques demonstrate that MT-108, a potent and selective α4β7 inhibitor, impairs B cell trafficking by effectively blocking B cell adhesion in the Peyer's patches.
- The MT-108 mediated decrease in cell adhesion to endothelial cells correlated with a significant increase in B cell rolling velocity and flux.
- MT-108 impacted B cell trafficking to a similar degree as the anti-α4β7 blocking antibody DATK32.
- An α4β7 SMi can rapidly impact cell homing to the gut, fully replicating the speed of onset and efficacy of an antibody.

**References:** [1]Redhu et al., 2022 (DDW), [2]Canales-Herrerias et al., 2024.
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**Disclosures:** A.S., N.S.R., D.L., D.G., M.R., B.H., M.B., B.G., B.R., and J.W. were employed by Morphic Therapeutic for the duration of the study.

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