The development of a gut-specific immune response to an oral antigen is dependent on α4β7 mediated lymphocyte trafficking

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INTRODUCTION

The inhibition of α4β7 integrin is a clinically-validated therapeutic target for the effective treatment of inflammatory bowel disease (IBD). The generation of an immune response to an oral antigen begins at inductive sites such as Peyer's patches followed by cell migration to effector sites in the gut. α4β7 integrin, which binds to MAdCAM-1, is known to play a major role in cell trafficking to gut-associated lymphoid tissues. Vedolizumab, an anti-α4β7 mAb, has been shown to reduce the antibody response to an oral cholera vaccine, but not to an intramuscularly delivered hepatitis B vaccine in healthy subjects (Wyant et al., 2015). In the current study, we investigated whether MT-102, a potent and selective small molecule inhibitor of α4β7 has similar effects on oral versus non-mucosal immunizations to those reported in the clinic for vedolizumab.

METHODS

Oral Cholera Toxin (CTX) Immunization Model: An *in vivo* immunization model was established to evaluate the generation of a gut-specific immune response to CTX. BALB/c mice were first immunized subcutaneously with CTX to prime the immune system followed by weekly CTX oral immunizations. Mice receiving MT-102 were implanted with osmotic minipumps prior to CTX immunizations. Alternatively, mice were treated 3x/week with the anti-α4 mAb (PS/2). Fecal samples were collected every week while blood and Peyer's patches (PP) were collected at the end of the study. MT-102 drug levels were quantified by mass spectrometry.

Figure 1: Schematic for the induction of a gut specific immune response.

Total and anti-CTX IgA/IgG ELISA: To quantify anti-CTX antibodies in fecal and plasma samples, plates were coated overnight with 2 µg/ml CTX. Mouse IgA and IgG ELISA kits (Invitrogen) were used to quantify anti-CTX respective antibody titers. The mouse IgA kit was also used to quantify total fecal IgA. To measure anti-NP-KLH IgG the plates were coated with 10 µg/ml NP-KLH.

Peyer's patches FACS Analysis: PPs were isolated and processed into a single cell suspension. For FACS analysis, cells were stained for viability, B220, and CD3. The samples were acquired on a Bio-Rad ZE5 flow cytometer and data were analyzed using FlowJo software.

Non-Mucosal Immunization Model: BALB/c mice were immunized with the hapten 4-Hydroxy-3-nitrophenylacetyl conjugated to Keyhole Limpet Hemocyanin (NP-KLH). Mice were treated 3x/week with anti-MAdCAM-1 mAb (MECA-367) or with an isotype control. Blood and fecal samples were collected during the study. A rat IgG2a ELISA kit (Invitrogen) was utilized to measure MECA-367 and isotype levels in the plasma.

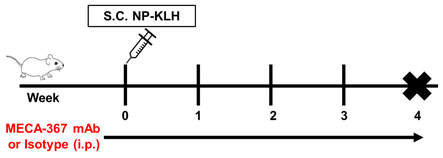
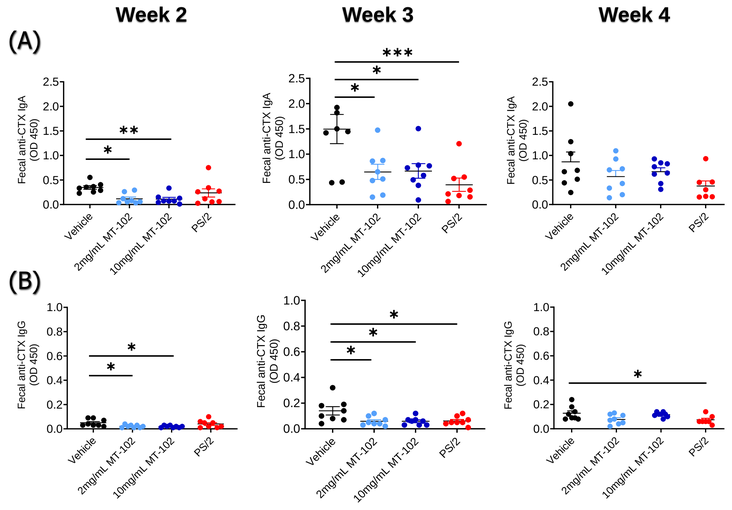


Figure 2: Schematic for the generation of a systemic immune response to a non-mucosal antigen.

RESULTS

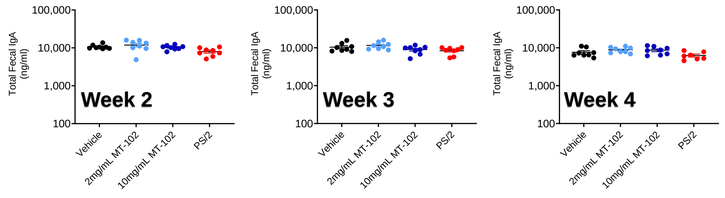
Oral Immunizations

Figure 3: α4β7 inhibition delays the production of anti-CTX IgA and IgG antibodies in the gut.



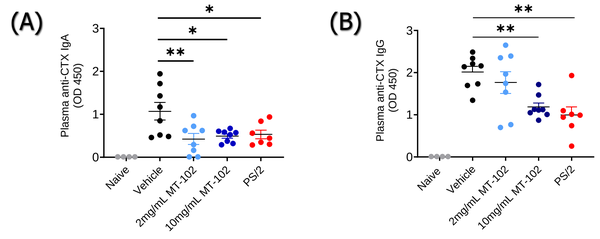
Anti-CTX IgA (A) and IgG (B) antibodies were quantified by ELISA from weeks 2-3 (1:10 dilution) and week 4 (IgA-1:50, IgG-1:10) samples from animals treated as depicted in Figure 1. Data are mean ± SEM, analyzed by one-way ANOVA with Dunnett's multiple comparisons test, ***p <0.001, **p <0.01, *p <0.05 relative to vehicle control (n = 7-8 per group).

Figure 4: Total gut IgA levels are unaffected by α4β7 inhibition.



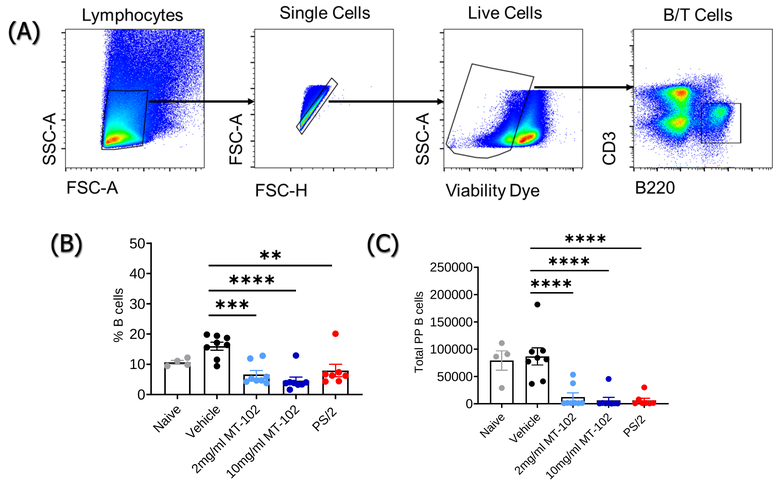
Total fecal IgA antibodies in CTX immunized mice as depicted in Figure 1. Data are mean ± SEM, analyzed by one-way ANOVA with Dunnett's multiple comparisons test (n = 7-8 per group).

Figure 5: Plasma anti-CTX IgA and IgG antibodies were reduced by α4β7 blockade.



Anti-CTX IgA (A) and IgG (B) antibodies were measured in plasma samples from week 4 animals treated as depicted in Figure 1. Data are mean ± SEM, analyzed by one-way ANOVA with Dunnett's multiple comparisons test, **p <0.01, *p <0.05 relative to vehicle control (n= 4-8 per group).

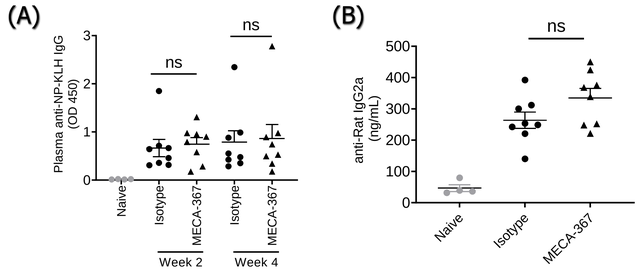
Figure 6: The inhibition of α4β7 decreased B cells in the PPs.



A) Representative flow cytometry plots showing the gating strategy for PP B cells. The frequency (B) and total number (C) of B cells in MT-102 and PS/2 treated mice. Data are mean ± SEM, analyzed by one-way ANOVA with Dunnett's multiple comparisons test, ****p <0.0001, ***p <0.001 **p <0.01, relative to vehicle control (n = 7-8 per group).

Non-Mucosal Immunization

Figure 7: Anti-MAdCAM-1 antibody MECA-367 has no effect in the generation of a systemic IgG response to NP-KLH.



(A) Anti-NP-KLH IgG antibodies in the plasma of immunized mice as depicted in Figure 2. (B) The concentration of both isotype and MECA-367 rat IgG2a antibodies found in the plasma of mice at the end of the study. Data are represented as mean ± SEM, analyzed by t-test (n=8 per group).

CONCLUSION

- MT-102 delays the production of anti-CTX antibodies in the gut without perturbing total IgA levels.
- Inhibition of α4β7-mediated trafficking specifically inhibits the response to an oral, but not to a non-mucosal, antigen.
- MT-102, a potent and selective α4β7 inhibitor, replicates the pharmacology of an antibody.