Inflammatory Bowel Diseases, 2025, **31**, 290–293
https://doi.org/10.1093/ibd/izae023
Advance access publication 9 February 2024
Brief Report - Clinical

Vedolizumab Does Not Affect Antibody Secreting Cell Recruitment to the Lactating Mammary Gland of Mothers With Inflammatory Bowel Disease

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Lay Summary

Despite a known role for α4β7 and MAdCAM-1 for the recruitment of antibody secreting cells to the lactating mammary gland, vedolizumab which targets integrin α4β7 did not lower breastmilk IgA in lactating mothers with IBD receiving the drug. It is likely that antibody secreting cells alternatively employ α4β1 to arrest on VCAM-1 also expressed by the lactating mammary gland.

Key Words: vedolizumab, integrins, IgA, lactation

Abbreviations: vdz, vedolizumab; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; breastmilk, BM; Ig, immunoglobulin; SIgA, secretory immunoglobulin A; SIgM, secretory immunoglobulin M; ASC, antibody secreting cells; MG, mammary gland; GI, gastrointestinal; GALT, gastrointestinal associated lymphoid tissue; LMG, lactating mammary gland; MAdCAM-1, mucosal addressin cell adhesion molecule-1; VCAM-1, vascular addressin cell adhesion molecule

Introduction

Diarrheal and respiratory infections are the main causes of infant mortality worldwide. Passive transfer of maternal antibodies in breast milk (BM) provides the first line of defense against these during the neonatal period. There is ample evidence dating back to the 1960s that breastfeeding decreases childhood morbidity and mortality. A systematic review of 18 clinical studies found a significant positive effect of breastfeeding against incidence, prevalence, hospitalization, and mortality from diarrheal diseases. The degree of protection was dependent on the level of breastfeeding exposure. This supports current WHO recommendation for exclusive breastfeeding during the first 6 months of life, as a key child survival intervention.[1] Breast milk contains multiple bioactive protective factors, amongst the most important are secretory immunoglobulins (Igs): secretory (S)IgA and SIgM.[2] Of these, SIgA represents over 90% of milk Igs. Given the high risk posed to infants by pathogens encountered at mucosal surfaces, most milk SIgA/M is targeted to common gastrointestinal (GI) and respiratory pathogens.

To be imprinted with specificity against intestinal pathogens, antibody secreting cells (ASCs) recruited to the mammary gland (MG) are educated at gastrointestinal-associated lymphoid tissue (GALT; Figure 1A), thus they predominantly express integrin α4β7 and the chemokine receptor CCR10. The microvasculature of the lactating MG (LMG) in turn expresses the α4β7-ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and the chemokine CCL28, solely before and during the lactation period[3] (Figure 1B). Given the known extraintestinal expression of MAdCAM-1 by the LMG, we investigated whether blockade of its counterpart integrin α4β7 by vedolizumab (vdz) blocked recruitment of IgA ASCs into LMG of mothers with IBD, reflected by decreased SIgA and SIgM levels in BM, as it is established that all Igs present in milk are produced by resident ASCs within the gland.[3-6]

Materials and Methods

Source of Samples

Breast milk samples were provided by breastfeeding women aged 18 and older who resided in the United States or Canada and were enrolled in the UC San Diego Human Milk Research Biorepository (HMB). Each participant provided written consent, completed a maternal interview to collect demographics, details regarding lactation, maternal and child health history, and specific information regarding medication and other exposures for the 14 days prior to milk sample collection. Human milk samples were collected using a standardized protocol that has been described previously.[7] The study was approved by the UC San Diego Institutional Review Board.

Received for publication: December 7, 2023. Editorial Decision: January 21, 2024

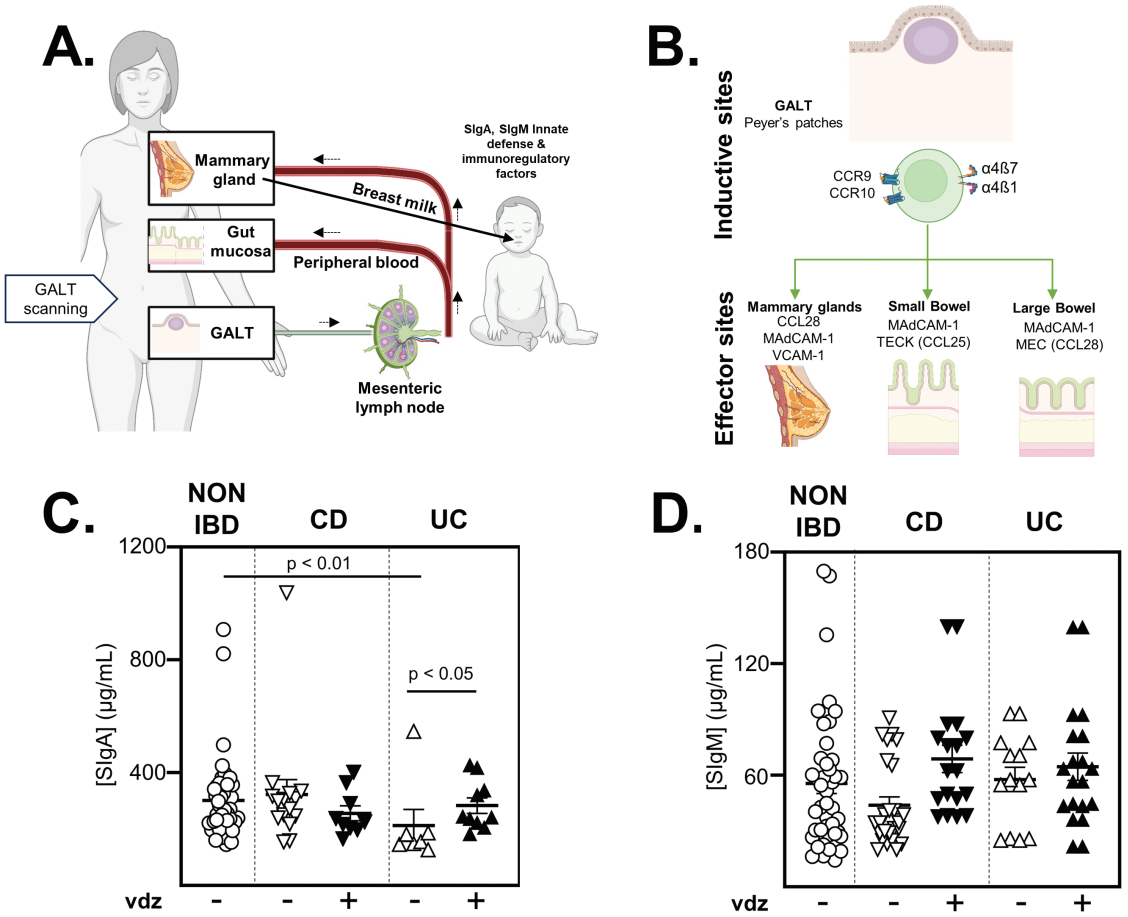

Figure 1. Traffic of ASC from GALT to LMG, critical molecules involved and SIgA/SIgM levels of BM from mothers with and without IBD who were treated or untreated with vdz. A, ASC educated in the GALT, traffic to the mesenteric lymph nodes, and circulation to populate the LMG. B, α4β7,α4β1, CCR10 are expressed by ASC migrating to the LMG microvasculature expressing MAdCAM-1, VCAM-1 and CCL28. C, SIgA and D, SIgM levels of BM from normal controls (NON IBD, $n = 45$), mothers with IBD not receiving (CD, vdz $n = 15$; UC, vdz $n = 7$) or receiving vedolizumab (CD + vdz $n = 9$; UC + vdz $n = 10$) were measured by ELISA and presented as mean ± SD. Lower milk SIgA levels were observed only in mothers with UC not treated with vdz compared with non-IBD controls ($P < .01$) and vdz-treated mothers ($P < .05$) matched by day of lactation after birth.

Study Subjects

Participants were selected based on treatment for IBD including Crohn's disease (CD) and ulcerative colitis (UC). Participants ranged in age from 32 to 41 years, with body mass index (BMI) from 25 to 31. Mothers with IBD had been diagnosed between 8 and 18 years prior to sample collection, and those receiving vdz had received the drug by IV infusion every 8 weeks for at least 18 months prior (Table 1). Two of the 19 vdz-treated donors received the drug every 4 weeks. Information regarding disease activity at time of milk collection was not available. Milk was collected between 1 and 15.5 months after childbirth. Comparison subjects were either normal controls or mothers with IBD not treated with vdz. None of the participating mothers reported mastitis up to 14 days prior to milk collection.

Immunoglobulin Determination by ELISA

The Ig levels were determined in samples of cryopreserved BM from mothers without IBD ($n = 45$) in vdz-treated mothers diagnosed with either UC ($n = 10$) or Crohn's ($n = 9$) and in mothers with UC ($n = 7$) or CD ($n = 15$) not treated with vdz, using commercial ELISA Kits (IgA: cat #88-50600-88, IgM: cat #88-50620-88, Thermo Fisher Scientific, Waltham, MA, USA) as per manufacturer's instructions. To address potential fluctuation of Ig levels based on postpartum timing, we tested milk samples from non-IBD mothers for each of the vdz-treated or untreated mothers with IBD, matched by lactation day postdelivery. Samples were diluted at 1:30 000 for IgA analysis and at 1:200 for IgM analysis. ELISA results were read using a BioTek Synergy Mx Microplate Reader (BioTek, Winooski, VT, USA) and analyzed using the BioTek Gen5 software (BioTek).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software Inc, La Jolla, CA, USA). Column statistics tests were used to assess parametric distribution of data sets. For comparison of 2 groups, the nonparametric Mann-Whitney test was utilized. Descriptive statistics were displayed as mean ± standard deviation in the table and figure. Significance was defined as P values of <0.05 and resulting statistically significant difference as indicated.

Table 1. Maternal characteristics and breastmilk Ig levels.

Groups	Month of Lactation	Maternal Age (years)	Maternal BMI	Disease Duration (years)	Concomitant Medication	SIgA µg/mL	SIgM µg/mL
non-IBD controls for CD (n = 24)	3 ± 2	35 ± 6	25 ± 6	N/A	N/A	292 ± 153	51 ± 33
CD—vdz (n = 15)	4 ± 4	33 ± 3	25 ± 4	9 ± 6	anti-TNF, ustekinumab, 6-MP, Prednisone	322 ± 207	46 ± 24
CD + vdz (n = 9)	3 ± 2	32 ± 3	25 ± 4	11 ± 6	5-ASA, 6-MP, tofacitinib	256 ± 80	69 ± 32
non-IBD controls for UC (n = 21)	6 ± 5	33 ± 5	25 ± 4	N/A	N/A	312 ± 143	61 ± 41
UC—vdz (n = 7)	5 ± 2	35 ± 5	25 ± 6	8 ± 5	anti-TNF, ustekinumab, 6-MP	212 ± 149	58 ± 26
UC + vdz (n = 10)	6 ± 5	34 ± 7	26 ± 5	14 ± 4	5-ASA	283 ± 87	65 ± 34

Results

Vedolizumab Did Not Affect BM SIgA nor SIgM Levels

As there was no significant variation between the control groups based on day of lactation, they are presented as a single group (NON IBD, Figure 1C). The levels of secretory IgA (SIgA) in breast milk of mothers without IBD were not different from those of mothers with CD, whether or not they were untreated (vdz) or those treated with vdz (vdz+). Unexpectedly, milk SIgA from mothers with UC not on vdz (n = 7) was lower than non-IBD controls and those from mothers with UC on vdz (Figure 1C). No difference in SIgM levels were seen between mothers without IBD and with UC or CD whether or not they were on vdz (Figure 1D).

Discussion

Here we report that treatment with vdz does not affect Ig levels in BM of mothers with IBD. In adult mammals, MAdCAM-1 is expressed in the intestine and GALT constitutively during homeostatic conditions. It is also inducible and increased during inflammation at extraintestinal sites, such as in the inflamed pancreas, liver, and genitourinary tract.[8] In the LMG microvasculature, MAdCAM-1 is induced in the absence of inflammation under hormonal stimuli, such as progesterone, prolactin, etc.[9] Induction of MAdCAM-1 in mouse LMG predates birth by around 14 days, coinciding with the major influx of IgA ASCs,[10,11] which correlate with increasing IgA levels in BM.[11,12] This is consistent with an important role of the α4β7/MAdCAM-1 axis in the process. However, while MAdCAM-1 expression decreases after birth, IgA ASC recruitment continues unabated, supporting an alternate pathway such α4β1/VCAM-1 for continuous ASC recruitment after birth.[10,11] This could be particularly the case for ASC primed at pulmonary sites against respiratory pathogens. The chemokine/receptor CCL28/CCR10 axis is also involved, and CCL28 antibody blockade in mice lowers milk SIgA.[13] However, to allow for the arrest of intestinal- or pulmonary-derived ASC from circulation, an activated integrin on the ASC must interact with its endothelial ligand on the LMG microvasculature. Additionally, VCAM-1 has been implicated in ASC recruitment in mice.[12] Indeed, VCAM-1 has been shown to be expressed in the LMG, although mostly in vessels of greater caliber than those that express MAdCAM-1.[5] In mice, the blockade of VCAM-1 but not MAdCAM-1 decreased milk IgA, leading to the conclusion that MAdCAM-1 was less important in the process. However, careful examination of the experimental methods shows that MAdCAM-1 blockade was started after birth, therefore not interfering with peak MAdCAM-1-mediated ASC recruitment, which predates it.[5] It is likely that to assess the role of MAdCAM-1 in the process, blockade should have begun no less than 2 weeks before birth, where most IgA ASC are being recruited to LMG, in preparation for birth and onset of lactation for passive immunity.

Vedolizumab is detectable at low levels in BM of mothers with IBD receiving the drug. However, there was no increase in gastrointestinal or any other kind of infections up to 10 months of age.[14] Our study found no effect of vdz treatment on BM SIgA/M levels, consistent with a redundant role of the α4β7/MAdCAM-1 pathway for the recruitment of gut-derived ASC to the mammary gland. Unpublished results from our laboratory have shown both single and double expression of both α4β7 andα4β1 integrins on most cells in the periphery, intestinal lamina propria and GALT of mice and humans. We propose that the lower IgA levels observed in patients with UC not receiving vdz is likely due to the small sample size, which is a limitation of the study. This is further supported by similar levels of SIgM in all study subjects, as IgM ASCs would use the same integrins to reach the LMG. The lack of information regarding disease activity at time of collection is an additional study limitation, particularly if BM Ig levels would have been different between mothers with and without IBD. A prior study found lower IgA levels in BM of mothers with UC and CD. On average, these mothers had higher fecal calprotectin levels than the controls, suggesting that inflammation might decrease BM IgA.[15]

It is conceivable that sustained α4β7 blockade by vdz leads to adaptations in the recruitment process, allowing or enhancing ASC recruitment mediated by the α4β1/VCAM-1 pathway. Alternatively, ASC primed at respiratory sites, thus not utilizing α4β7/MAdCAM-1, compensate for GALT-primed cells in the LMG, correcting an overall IgA deficit. If our hypothesis is true, then natalizumab, which targets both α4 integrins, may affect ASC recruitment and lower milk SIgA/M. It is reassuring that despite the established role of the α4β7/MAdCAM-1 axis for ASC recruitment to

the LMG, passive immunity from BM appears to be unaffected by sustained vdz blockade of the α4β7 integrin. Larger epidemiologic studies support the safety of vdz during pregnancy and neonatal period.[14,16,17] Whether vdz might still interfere with ASC primed in the intestine, rather than in the lung, without a net effect on total IgA, could be addressed by examining the intestinal vs respiratory specificities of the SIgA/M in vdz-treated mothers using methodologies that have been reported recently.[18] So far, there is no evidence that infants or children whose mother are receiving vdz are at increased of gastrointestinal infections or any kind. Review of the data published so far supports that either α4 integrin α4β7 or α4β1 interacting with either MAdCAM-1 or VCAM-1 lining LMG microvessels support sufficient ASC recruitment to maintain baseline SIgA levels in BM.

Author Contributions

J.R-N. and B.S.B. for study concept and design; J.U., T.M., C.C., K.B., B.S.B., and J.R-N. for data generation and review; J.U. and J.R-N. for analysis and interpretation of data and drafting the manuscript; C.C., B.B., T.M., and K.B. for critical review of the manuscript.

Funding

This work was supported by grants from the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK; R01DK32242, R01DK131532, K26DK138378), VA Merit BLRD-I01 BX005951 and San Diego Digestive Diseases Research Center (P30 DK120515).

Conflicts of Interest

Authors have nothing to disclose.

References

1. Lamberti LM, Fischer Walker CL, Noiman A, Victora C, Black RE. Breastfeeding and the risk for diarrhea morbidity and mortality. *BMC Public Health*. 2011;11(Suppl 3):S15.
2. Atyeo C, Alter G. The multifaceted roles of breast milk antibodies. *Cell*. 2021;184(6):1486-1499.
3. Brandtzaeg P. The mucosal immune system and its integration with the mammary glands. *J Pediatr*. 2010;156(2 Suppl):S8-15.
4. Bourges D, Meurens F, Berri M, et al. New insights into the dual recruitment of IgA+ B cells in the developing mammary gland. *Mol Immunol*. 2008;45(12):3354-3362.
5. Low EN, Zagieboylo L, Martino B, Wilson E. IgA ASC accumulation to the lactating mammary gland is dependent on VCAM-1 and alpha4 integrins. *Mol Immunol*. 2010;47(7-8):1608-1612.
6. Roux ME, McWilliams M, Phillips-Quagliata JM, Weisz-Carrington P, Lamm ME. Origin of IgA-secreting plasma cells in the mammary gland. *J Exp Med*. 1977;146(5):1311-1322.
7. Bandoli G, Bertrand K, Saoor M, Chambers CD. The design and mechanics of an accessible human milk research biorepository. *Breastfeed Med*. 2020;15(3):155-162.
8. Grant AJ, Lalor PF, Hubscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology*. 2001;33(5):1065-1072.
9. Weisz-Carrington P, Roux ME, McWilliams M, Phillips-Quagliata JM, Lamm ME. Hormonal induction of the secretory immune system in the mammary gland. *Proc Natl Acad Sci U S A*. 1978;75(6):2928-2932.
10. Tanneau GM, Hibrand-Saint Oyant L, Chevaleyre CC, Salmon HP. Differential recruitment of T- and IgA B-lymphocytes in the developing mammary gland in relation to homing receptors and vascular addressins. *J Histochem Cytochem*. 1999;47(12):1581-1592.
11. van der Feltz MJ, de Groot N, Bayley JP, et al. Lymphocyte homing and Ig secretion in the murine mammary gland. *Scand J Immunol*. 2001;54(3):292-300.
12. Boumahrou N, Chevaleyre C, Berri M, et al. An increase in milk IgA correlates with both pIgR expression and IgA plasma cell accumulation in the lactating mammary gland of PRM/Alf mice. *J Reprod Immunol*. 2012;96(1-2):25-33.
13. Wilson E, Butcher EC. CCL28 controls immunoglobulin (Ig)A plasma cell accumulation in the lactating mammary gland and IgA antibody transfer to the neonate. *J Exp Med*. 2004;200(6):805-809.
14. Mitrova K, Pipek B, Bortlik M, et al.; Czech IBD Working Group. Safety of ustekinumab and vedolizumab during pregnancy-pregnancy, neonatal, and infant outcome: a prospective multicentre study. *J Crohns Colitis*. 2022;16(12):1808-1815.
15. Meng X, Dunsmore G, Koleva P, et al. The profile of human milk metabolome, cytokines, and antibodies in inflammatory bowel diseases versus healthy mothers, and potential impact on the newborn. *J Crohns Colitis*. 2019;13(4):431-441.
16. Moens A, van der Woude CJ, Julsgaard M, et al. Pregnancy outcomes in inflammatory bowel disease patients treated with vedolizumab, anti-TNF or conventional therapy: results of the European CONCEIVE study. *Aliment Pharmacol Ther*. 2020;51(1):129-138.
17. Moens A, van Hoeve K, Humblet E, et al.; Belgian IBD Research and Development group (BIRD). Outcome of pregnancies in female patients with inflammatory bowel diseases treated with vedolizumab. *J Crohns Colitis*. 2019;13(1):12-18.
18. Johnson-Hence CB, Gopalakrishna KP, Bodkin D, et al. Stability and heterogeneity in the antimicrobiota reactivity of human milk-derived immunoglobulin A. *J Exp Med*. 2023;220(8):e20220839.