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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

CD36 Deficiency Impairs the Small Intestinal Barrier and Induces Subclinical Inflammation in Mice

Vincenza Cifarelli,[1] Stoyan Ivanov,[2] Yan Xie,[3] Ni-Huiping Son,[5] Brian T. Saunders,[2] Terri A. Pietka,[1] Trevor M. Shew,[1] Jun Yoshino,[1] Sinju Sundaresan,[1] Nicholas O. Davidson,[3] Ira J. Goldberg,[5] Andrew E. Gelman,[4] Bernd H. Zinselmeyer,[2] Gwendalyn J. Randolph,[2] and Nada A. Abumrad[1]

[1]Department of Medicine, Center for Human Nutrition, [2]Department of Pathology and Immunology, [3]Department of Medicine, Division of Gastroenterology, and [4]Department of Surgery, Washington University School of Medicine, St Louis, Missouri; and [5]Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, New York University Langone Medical Center, New York, New York

SUMMARY

This study documents that CD36 is important for intestinal homeostasis. CD36 deletion associates in the gut with altered extracellular matrix, neutrophil infiltration, and defective epithelial barrier. Systemically, the deletion results in subclinical inflammation with depletion of the Ly6C^{low} anti-inflammatory monocytes. Specific loss of endothelial cell CD36 recapitulates most intestinal phenotypes of germline CD36KO mice.

BACKGROUND & AIMS: CD36 has immunometabolic actions and is abundant in the small intestine on epithelial, endothelial, and immune cells. We examined the role of CD36 in gut homeostasis by using mice null for CD36 (CD36KO) and with CD36 deletion specific to enterocytes (Ent-CD36KO) or endothelial cells (EC-CD36KO).

METHODS: Intestinal morphology was evaluated by using immunohistochemistry and electron microscopy. Intestinal inflammation was determined from neutrophil infiltration and expression of cytokines, toll-like receptors, and cyclooxygenase-2. Barrier integrity was assessed from circulating lipopolysaccharide and dextran administered intragastrically. Epithelial permeability to luminal dextran was visualized by using two-photon microscopy.

RESULTS: The small intestines of CD36KO mice fed a chow diet showed several abnormalities including extracellular matrix accumulation with increased expression of extracellular matrix proteins, evidence of neutrophil infiltration, inflammation, and compromised barrier function. Electron microscopy showed shortened desmosomes with decreased desmocollin 2 expression. Systemically, leukocytosis and neutrophilia were present together with 80% reduction of anti-inflammatory Ly6C^{low} monocytes. Bone marrow transplants supported the primary contribution of non-hematopoietic cells to the inflammatory phenotype. Specific deletion of endothelial but not of enterocyte CD36 reproduced many of the gut phenotypes of germline CD36KO mice including fibronectin deposition, increased interleukin 6, neutrophil infiltration, desmosome shortening, and impaired epithelial barrier function.

CONCLUSIONS: CD36 loss results in chronic neutrophil infiltration of the gut, impairs barrier integrity, and systemically causes subclinical inflammation. Endothelial cell CD36 deletion reproduces the major intestinal phenotypes. The findings suggest an important role of the endothelium in etiology of gut inflammation and loss of epithelial barrier integrity. *(Cell Mol Gastroenterol Hepatol 2017;3:82–98; http://dx.doi.org/10.1016/j.jcmgh.2016.09.001)*

Keywords: Neutrophils; Endothelium; Fibronectin; Collagen.

The scavenger receptor CD36 mediates intracellular signaling in response to its ligands in a cell-type and context-dependent manner.[1] In the small intestine, CD36 is abundantly expressed on enterocytes, endothelial cells, and immune cells. CD36 present on the apical membrane of enterocytes recognizes dietary long-chain fatty acids and cholesterol and is important for chylomicron production.[2] CD36 null (CD36KO) mice have delayed intestinal lipid absorption, resulting in more fat reaching the distal gut, and produce smaller chylomicrons that are slowly cleared from the circulation.[3,4] The absorptive intestinal epithelium functions as a tight barrier limiting entry of pathogens and related toxins such as lipopolysaccharides.[5] Recruitment of immune cell to the gut elicits the acute inflammation necessary for host defense and later contributes to inflammation resolution and tissue healing.[6,7] CD36's role in gut immunity is unknown, but on monocytes/macrophages CD36 functions in recognizing pathogen-associated and

Abbreviations used in this paper: **COX-2, cyclooxygenase 2; ECM, extracellular matrix; FITC, fluorescein isothiocyanate; IL, interleukin; MPO, myeloperoxidase; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SEM, standard error of the mean; α-SMA, smooth muscle actin alpha; TLR, toll-like receptor; TNF, tumor necrosis factor; TUNEL, deoxyuride-5'-triphosphate biotin nick end labeling.**

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danger-associated molecular pattern molecules that can initiate and sustain inflammatory responses.[8,9] CD36 also participates in resolution of inflammation by inducing polarization of macrophages to the anti-inflammatory M2 phenotype important for phagocytic clearance of apoptotic neutrophils[10] and tissue healing.[11,12] On endothelial cells, CD36 functions as a receptor for extracellular matrix (ECM) components such as thrombospondin 1 and collagen and acts as a signaling platform for several integrins.[13,14] Interaction of immune cells with the ECM is crucial to their activation and survival.[15,16] ECM dysregulation promotes tissue inflammation and might contribute to etiology of inflammatory bowel disease.[17]

The range of functions for intestinal CD36 (absorption, immunity, ECM) suggests it could play an important role in intestinal homeostasis, but this remains unexplored. In the present study, we examined the impact of CD36 deletion on intestinal barrier integrity and inflammation in mice fed a chow diet under basal conditions and after administration of a fat bolus. We show that germline CD36 deletion results in abnormal ECM remodeling, defective epithelial barrier, and neutrophil infiltration in the proximal intestine. Systemically, the deletion associates with endotoxemia and subclinical inflammation. Specific deletion of endothelial CD36 also results in a leaky epithelium and gut neutrophil infiltration, supporting a primary role in eliciting the gut abnormalities of the germline CD36KO mouse.

Materials and Methods

Animals

All studies followed guidelines of the animal ethics committee of Washington University School of Medicine (St Louis, MO). CD36-null (CD36KO), enterocyte null (Ent-CD36KO), endothelial null (EC-CD36KO), and wild-type (WT) mice, all on the C57BL/6 background, were housed in a facility with a 12-hour light-dark cycle and fed chow ad libitum (Purina, St Louis, MO) or when indicated fasted for 12 hours with ad libitum access to water. CD36 floxed (Fl/Fl) mice were generated by using a plasmid with LoxP sites flanking CD36 exons 2 and 3. After electroporation, selection, and screening, properly targeted ES clones were injected into blastocyst for generation of chimeric mice. Mice carrying the CD36 floxed allele were crossed with C57BL6 mice expressing Cre recombinase driven by the villin promoter (B6.Cg-Tg(Vil-cre)997Gum/J, stock number 021504; Jackson Laboratory, Bar Harbor, ME) to disrupt CD36 expression in enterocytes (Ent-CD36KO).

To delete endothelial CD36 (EC-CD36KO), CD36 floxed mice were crossed to C57BL6 mice expressing Cre driven by the Tie2 promoter. To avoid germline transmission, breeding involved Cre+ males with Cre– females. Genotypes were confirmed by polymerase chain reaction and immunohistochemistry. Endothelial cells were isolated from lungs of Fl/Fl and EC-CD36KO mice by using CD146 MicroBeads (Miltenyi Biotech, Cambridge, MA; cat. #130-092-007), and RNA from ~500,000 $CD146^+$ cells was used to measure CD31 and CD36 mRNA levels by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

For the bone marrow chimera experiments, B6.SJL-$Ptprc^aPep3^b$/BoyJ mice (Jackson Laboratories) with the CD45.1 allelic version of the CD45 leukocyte common antigen were irradiated (1100 rad by using a cesium source) and injected retro-orbitally with the appropriate WT or CD36KO bone marrow suspension (2×10^6 cells) as previously described.[18] Blood and intestines were collected 8 weeks later for flow cytometry and immunohistochemistry.

Intestine Permeability and Endotoxin Measurement

Fluorescein isothiocyanate (FITC)–conjugated dextran (4 kDa) (Sigma-Aldrich, St Louis, MO) was intragastrically administered to mice (n = 6/genotype) sedated initially with 4%–5% isoflurane and maintained with 1%–2% isofluorane. Blood was collected retro-orbitally at 0, 2, 4, and 6 hours, and level of FITC-dextran was measured at excitation 485/20 and 528/20 emission (Synergy HT; BioTek Instruments Inc, Winooski, VT). One week later, the experiment was repeated by using the same mice groups, but a bolus of triolein (4.5 μL/g body weight) was administered intragastrically 30 minutes before the FITC-dextran. For endotoxin determinations, sera were diluted 1:20, heated (70°C, 15 minutes), and assayed by using the limulus amebocyte lysate chromogenic endotoxin quantification (Lonza Inc, Walkersville, MD).

Histology and Immunohistochemistry

Small intestines were gently washed in cold phosphate-buffered saline (PBS), opened longitudinally, fixed in 10% formalin, and paraffin embedded. Sections (5 μm) were deparaffinized, followed by antigen retrieval (99°C, 18 minutes) in a pressurized chamber (Biocare Medical, Concord, CA) and blocked (1 hour) in donkey serum (2%) (Jackson Laboratories) and bovine serum albumin (3%) (Sigma-Aldrich). Incubation with primary antibodies (Table 1) was performed overnight (4°C), followed by incubation with horseradish peroxidase (Jackson Immuno Research Laboratories, West Grove, PA) or fluorescently labeled secondary antibodies (1 hour) (Alexa Fluor; BD Bioscience, San Jose, CA). For bone marrow staining, mouse femurs were fixed with 4%

Table 1. List of Antibodies, Source, and Dilution Used

Antigen	Host	Source	Dilution
MPO	Rabbit	Abcam	1:200
TLR2	Mouse	R&D Systems	1:100
Collagen 1α	Rabbit	Abcam	1:250
Fibronectin	Rabbit	Abcam	1:250
α-SMA	Rabbit	Sigma-Aldrich	1:500
CD36	Goat	R&D Systems	1:100
CD31	Mouse	BD Bioscience	1:500

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