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Loss of Acid Ceramidase in Myeloid Cells Restores Sphingolipid Composition to Alleviate Chronic Colitis in IL10-Deficent Mice

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Background: Inflammatory bowel disease (IBD) is characterized by chronic inflammation in the colon and increases the risk of colitis-associated colon cancer (CAC). Sphingolipids are a class of bioactive lipids that have been implicated in numerous diseases including IBD and cancer. Acid ceramidase (AC) is known to be overexpressed in IBD and CAC. AC degrades ceramide and leads to the generation of sphingosine-1-phosphate (S1P) which promotes immune cell recruitment, inflammation, and survival of potentially cancerous cells. Our lab has previously shown that loss of AC in myeloid cells (AC^{MYE}) significantly attenuates acute colitis and CAC. However, the impact of AC in chronic colitis is not known. To assess the role of AC in chronic colitis we generated $AC^{MYE}IL10^{-/-}$ and $AC^{fl/fl}IL10^{-/-}$ mice, which spontaneously develop chronic colitis and to better model human disease.

Objectives: (1) Define the role of AC in myeloid cell sphingolipid metabolism in chronic colitis, and (2) determine the impact of myeloid cell AC in immune cell recruitment.

Methods: $AC^{MYE}IL10^{-/-}$ and $AC^{fl/fl}IL10^{-/-}$ mice were generated and examined at 8 and 24 weeks of age. Male and female mice were utilized to investigate sex differences. Colons were frozen for realtime-rtPCR, ELISAs, and sphingolipid analysis or were formalin fixed for histology. Serum was collected and frozen for ELISAs. Blood, spleen, and colon tissues were assessed by flow cytometry.

Results: $AC^{MYE}IL10^{-/-}$ mice were protected from classical parameters of colitis including spleen enlargement and loss of colon length at 24 weeks of age. Sphingolipids were analyzed using LC-MS/MS to determine the impact of AC loss in myeloid cells on total colon sphingolipids. Total ceramides and sphingomyelins (SMs) were elevated in $AC^{MYE}IL10^{-/-}$ mice at 8 weeks of age. Long chain ceramides (C14, C16, C18:1) were increased in $AC^{MYE}IL10^{-/-}$ mice at 8 or 24 weeks of age. Interestingly lyso-SM, which can be generated by via AC, was reduced in $AC^{MYE}IL10^{-/-}$ mice. In addition, sphingoid bases, dhS1P and S1P were also reduced in $AC^{MYE}IL10^{-/-}$ mice. Realtime rtPCR and ELISAs were used to determine local inflammatory responses in colon tissues. mRNA expression of proinflammatory markers significantly increased in $AC^{fl/fl}IL10^{-/-}$ mice from 8 to 24 weeks of age and were reduced in $AC^{MYE}IL10^{-/-}$ mice at 24 weeks of age. Furthermore, protein expression of CCL5 and IL-1β were significantly reduced in colon tissues of $AC^{MYE}IL10^{-/-}$ mice at 24 weeks of age. Flow cytometry was used to assess the role of AC in immune cell recruitment. Neutrophil and type M1 macrophage populations were significantly reduced in the lamina propria (LP) and intra-epithelial layer (IEL) compartments in $AC^{MYE}IL10^{-/-}$ colons. In addition, tolerogenic CD11c+ dendritic cells were increased in the LP of $AC^{MYE}IL10^{-/-}$ mice. Effector T cell populations and surface expression of effector markers were reduced in both the LP and IEL.

Conclusion: Collectively these data suggest that loss of AC in myeloid cells may protect from chronic inflammation by reducing sphingoid bases, and reducing recruitment or maturation of inflammatory immune cell populations. These data identify the potential for AC as a therapeutic target for patients with IBD to reduce inflammation and the development of cancer.

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