RESEARCH LETTER

C1qa Muscularis Macrophages Regulate Gastrointestinal Motility Through Close Association With Enteric Neurons

The 1qa, 1qb, and 1qc proteins belong to the complement family, which serves in the classic immune cascade that physically defends against invading pathogens. More recently, C1qa has been found to play essential roles in keeping the homeostasis in the central nervous system (CNS) by regulating synapse pruning.[1] Tissue macrophages, such as microglia in the CNS, can produce a large amount of C1qa that reduces endogenous material (apoptotic cell debris) accumulation and preserves a proper immune response.[2] Indeed, in the CNS, microglia, but not neurons or peripheral blood, are the primary source of C1qa.[3] In the gastrointestinal (GI) tract muscularis propria, a subpopulation of tissue-resident muscularis macrophages (MMs)[4] shares phenotypic similarities with microglia.[5] The gut muscularis propria is also home to enteric neurons (ENs), which are organized in an intricate system of ganglia to control intrinsic GI functions,[6] and MM-EN functional connections can modulate GI motility.[7] However, there is no information on whether C1qa is present and functional in GI MMs. Therefore, this study aimed to determine the distribution of C1qa on MMs and whether C1qa MMs affect GI motility using a mouse model in which C1qa was selectively removed from macrophages.

We first asked whether MMs expressed C1qa in different gut regions. We isolated $CD11b^+$ cells from the stomach, small intestine, and colon to compare C1qa expression levels between $CD11b^+$ and $CD11b^-$ cells (Figure 1A and B). C1qa expression in $CD11b^+$ cells was higher than $CD11b^-$ cells ($P < .01$, Figure 1B), showing that MMs are the primary source of C1qa in the gut. In addition, C1qa expression levels were higher in the colon, followed by the stomach and the small intestine (Figure 1B). MMs constitute a heterogeneous population in which cells of different origins and functions coexist.[8] Thus, we next looked by immunohistochemistry at whether C1qa was expressed by subpopulations of MMs or by the entire tissue resident MMs pool using Major Histocompatibility Complex Class II, a general myeloid cell marker.[7] We found that not all $MHCII^+$ cells from different gut regions co-labeled with C1qa (Figure 1C) and only a few $C1qa^+$ cells in the stomach (arrow, Figure 1C) were not positive for MHCII.

MMs distribute across the different gut regions (Figure 1D) have a different morphology that may underline a different function.[9] $C1qa^+$ MMs were found in all regions of the gut muscularis propria (Figure 1D), although most of the $C1qa^+$MMs were located within the myenteric plexus. The colon and small intestine also showed many $C1qa^+$ MMs within the serosal region compared with the stomach (Figure 1E). In addition to II, C1qa co-labeled also with a more stringent macrophage marker, such as F4/80[10] (Figure A1). $C1qa^+$ microglia functionally interact with neurons to preserve CNS homeostasis.[1,3] Thus, we next looked at whether in the GI tract, C1qa MMs were closely associated with ENs. By whole-mount immunohistochemistry using a neuronal marker (β3-Tub), we distinguished the intraganglionic (blue, Figure 1F) from the interganglionic region and found C1qa labeling intensity higher in the intraganglionic region compared to the interganglionic (Figure 1G). We also see a dense network of $C1qa^+$ macrophages within the submucosal plexus (Figure A1C).

To study the contribution of C1qaMMs to GI motility, we used a $C1qa^{CKO}$ mouse model to deplete C1qa from myeloid cells. Indeed, compared to $C1qa^{FL}$, $C1qa^{CKO}$ mice demonstrated 80% reduction of C1qa expression in tissue (Figure A2A) and 95% in sorted $Cd11b^+$ cells (Figure 2A). MMs between the groups (Figure 2B, Figure A2B and C) were not changed, suggesting that C1qa is not required for regulation of MM density. $C1qa^{CKO}$ had faster whole gut transit (about 100 minutes) compared to $C1qa^{FL}$ ($P < .05$) (Figure 2C), but no differences to gastric emptying were observed between the groups (Figure 2D). We next measured the small bowel contribution to GI motility[11] and found that small bowel transit was accelerated in $C1qa^{CKO}$ compared to $C1qa^{FL}$ mice ($P < .01$, Figure 2E–F). Considering the differences in GI motility between males and females,[12] we next looked at whether C1qa expression and its functional role in GI physiology were altered in a sex-dependent manner. We found no changes in C1qa expression between males and females in the different gut regions (P = NS). In addition, we did not see any change in whole gut transit between male and females $C1qa^{CKO}$ mice (Figure A3A and B).

One limitation of the study is that mucosal macrophages also express C1qa[13] and therefore can affect GI physiology without necessarily interacting with ENs. The impossibility to distinguish between muscularis and mucosal macrophages using available genetic mouse models constitutes an obstacle for the whole field to

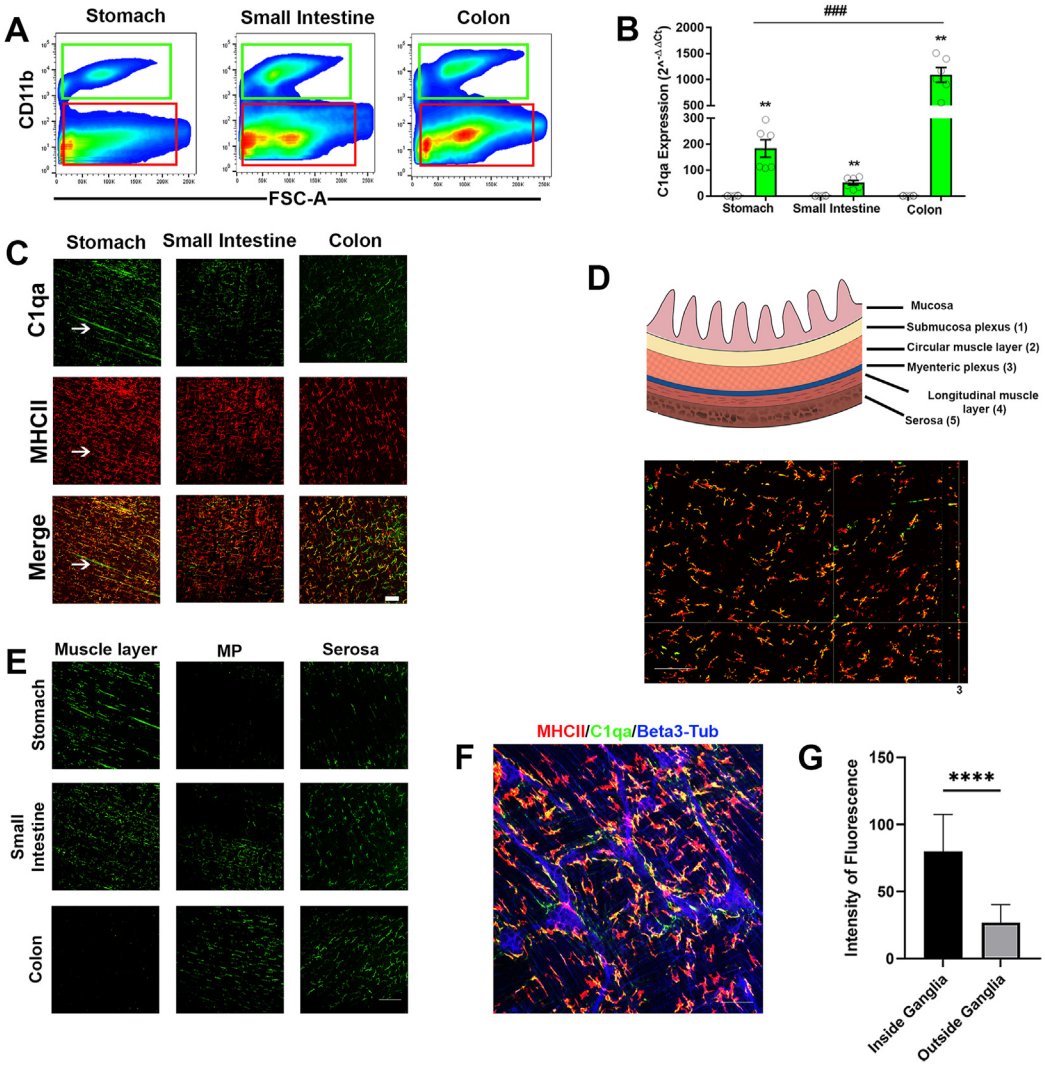

Figure 1. MMs are the main source of C1qa in the GI tract and C1qa MMs are in specific tissue regional cues. (A) Separation of $CD11b^{+}$ and $CD11b^{-}$ cells and (B) C1qa expression levels between $CD11^{+}$ and $CD11b^{-}$ cells from the 3 gut regions from the muscularis propria. (C) Whole-mount immunolabeling of MHCII and C1qa from the gut different regions of the muscularis propria. Arrows in the stomach and small intestine represent $C1qa^{+}/MHCII^{-}$ cells. Scale Bar: 100μm (D) Different layers of the muscularis propria. Orthogonal projections of C1qa and MHCII co-staining showing C1qa distribution within the different regions of gut muscularis propria. Lines identified a MM positive for MHCII and C1qa located in the myenteric plexus (3). Scale Bar: 40 μm. (E) C1qa labeling across the different regions of whole mounts projections of the muscularis propria. Scale Bar: 200μm. (F) Co-labeling of C1qa/MHCII/Beta3-tubulin within the myenteric plexus region. Scale Bar: 15μm. (G) Quantification of C1qa intensity labeling between MMs within the ganglia (intraganglionic, highlighted by the blue staining) and between the ganglia of colon (interganglionic). ****$P < .001$ by unpaired *t*-test.

selectively study the effect of MMs on GI motility.

In agreement with the recent study from Pendse and coworkers,[14] here we identified a novel population of MMs expressing C1qa distributed across the GI muscularis propria. This population is mainly associated with ENs at the level of the myenteric and submucosal plexus, and it contributes to GI motility. In the CNS, C1qa and the complement family, among various roles, are also essential for maintaining and regulating synapse formation. This study pointed out a new role for MMs, that in addition to maintaining ENs number,[8] can potentially regulate and maintain synapses as microglia in the CNS.[1] Understanding the mechanisms regulating C1qa MMs-ENs functional interaction and their involvement in functional diseases represent the next step toward targeting C1qa MMs for novel therapeutical strategies.

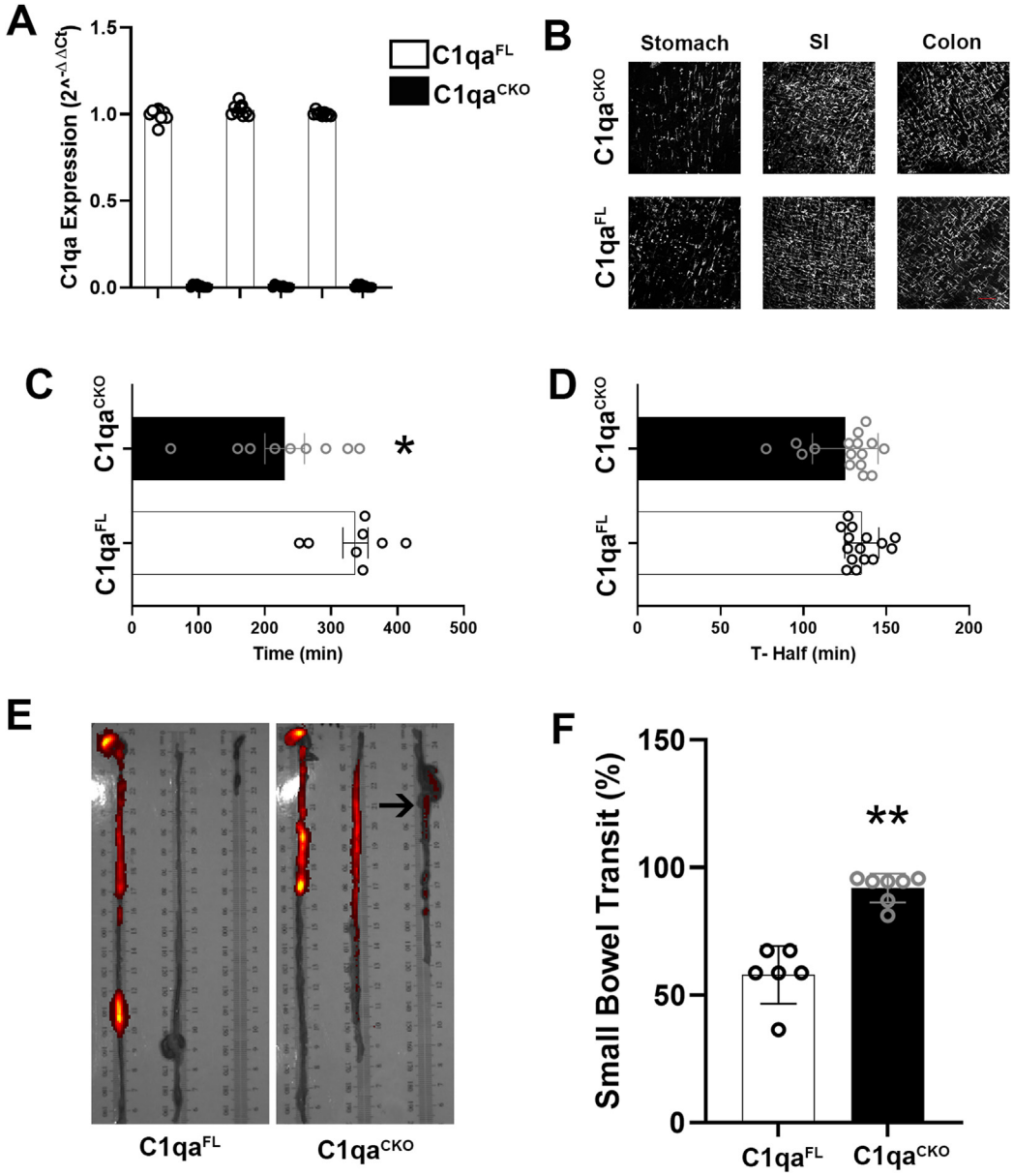

Figure 2. C1qa MMs regulate GI motility. (A) C1qa expression from the muscularis propria of the stomach, colon, and small intestine from CD11b+ cells between C1qa^{CKO} and C1qa^{FL} mice. (B) Muscularis propria whole-mount labeling and quantification of MHCII + cells between C1qa^{CKO} and C1qa^{FL} in the 3 gut regions. Scale Bar: 20 μm. (C) Whole gut transit time between C1qa^{CKO} and C1qa^{FL}. *$P < .05$ using unpaired *t*-test. (D) Gastric emptying between C1qa^{CKO} and C1qa^{FL}. (E) Small bowel transit using a fluorescent dye (rhodamine isothiocyanate (RITC)-dextran), administered by gavage, to evaluate the distribution of fluorescent contents across the GI tract after 30 minutes. Fluorescence intensity is overlayed and pseudo-colored to match the native fluorescence of gavaged material between C1qa^{CKO} and C1qa^{FL}. To note that in C1qa^{CKO} mice the dye intensity postgavaging was observed in the colon (arrow). (F) Representative image of small bowel transit. Data are expressed as mean ± SEM (n = 8). **$P < .001$ using a Mann-Whitney test. SEM, standard error of the mean.

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Supplementary Materials

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Abbreviations used in this paper: **CNS, central nervous system; ENs, enteric neurons; GI, gastrointestinal; MMs, muscularis macrophages**

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

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Data and analytic methods are presented in the article and in the Supplemental Information section. Additional data are available upon request from other researchers by contacting the corresponding author Dr Gianluca Cipriani (Cipriani.gianluca@mayo.edu).

Reporting Guidelines:
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